OCT 25 1949

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PLANT PHYSIOLOGY

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PUBLISHED QUARTERLY

BY

THE AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS

AT N. QUEEN ST. AND McGOVERN AVE., LANCASTER, PA.

Business Press, Inc.

Entered as second-class matter March 25, 1926, at the post office at Lancaster, Pa., under the Act of August 24, 1912.

Accepted for mailing at the special rate of postage provided for in paragraph 4, section 538. Authorized April 9, 1926.

PLANT PHYSIOLOGY

A QUARTERLY JOURNAL
DEVOTED TO ALL PHASES OF PLANT PHYSIOLOGY

Established 1926

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Subscription price, $10.00 a year. Single copies, $3.00 post free. Members of the American Society of Plant Physiologists receive the journal in connection with dues paid to the Society.

Missing numbers will be supplied if lost in the mail, provided that written notice is received within one month of the date of issue; otherwise they will be furnished at the single copy rate, as long as they are available.

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In Memoriam

OTIS FREEMAN CURTIS

1888–1949

OTIS FREEMAN CURTIS
1888–1949

OTIS FREEMAN CURTIS

1888–1949

Dr. Otis Freeman Curtis, Professor of Botany at Cornell University, died suddenly on July 4, 1949 while enjoying a brief vacation at Cape Cod, Massachusetts. He is survived by his wife, two sons, a daughter, six grandchildren and two sisters. His elder son, Dr. Otis Freeman Curtis, Jr., is a plant physiologist on the staff of the New York State Experiment Station at Geneva, New York. His younger son is assistant professor of zoology in St. Lawrence University, Canton, New York.

Dr. Curtis was born in Sendai, Japan on February 12, 1888 where his father, a minister of the Congregational Church, was active in missionary work. He left Japan at the age of seven and received his education in various parts of the United States finally entering Oberlin College in 1907 and receiving from Oberlin the A.B. degree in 1911. While at Oberlin he came under the influence of Professor Susan Percival Nichol, Professor of Botany in the institution, who undoubtedly stimulated young Curtis to enter the field of Botany. Dr. Curtis held a scholarship at Oberlin and during the summers of 1911 and 1912 the Oberlin Botanical Fellowship at Woods Hole.

Dr. Curtis entered Cornell in 1911 with his major in what was then the Department of Plant Physiology which in 1913 was merged with the newly created Department of Botany in the College of Agriculture at Cornell. He obtained the Ph.D. degree from Cornell in 1916.

In 1913, while still a graduate student, Curtis became an instructor in plant physiology. He continued in this position until July 1917 when he was made assistant professor, becoming Professor of Botany in 1922, and held this position until his death.

At Cornell teaching occupied a large share of Dr. Curtis' time but he, nevertheless, published about 30 papers dealing chiefly with vegetative reproduction, translocation, temperature and water relations of plants. His research work and monograph on translocation greatly stimulated research along new lines in this important field of work.

Dr. Curtis, with his students, also published a considerable number of papers dealing with translocation. His monograph on the subject brought together in one volume a critical appraisal of the status of the subject. He had also just finished a textbook on plant physiology with Professor D. G. Clark as junior author. He pioneered also in emphasizing the relation of reradiation of energy by plants and the relative non-importance of transpiration in controlling the temperature of plants.

Alternating for many years with Professor Lewis Knudson in an advanced course in plant physiology which ran throughout the year he taught between thirty to sixty graduate students who majored in the plant sciences

and soil technology. These men, now dispersed throughout the United States and in foreign countries in responsible positions, recall with pride their association with Dr. Curtis. Throughout his teaching he emphasized a critical appraisal of the subject. His examinations were not of the type to require solely a factual statement but of the kind to interpret plant response. He called also for critical considerations of published papers. As a result of these teaching methods, graduate students were heard to say of him during and after their days at Cornell, "He taught us to use our brains."

For a number of years Professor Curtis was Chairman of the Committee of the University Faculty which had just completed a report on the Improvement of Teaching in the University. In addition to serving on other important committees of the College and University, he was particularly interested in the Graduate School and for many years had been a member of the General Committee and for about five years was Secretary of the Graduate School.

Dr. C. W. deKiewiet, Acting President of Cornell University paid the following tribute to Dr. Curtis: "Many brilliant men have come into the world for a time, and have left in it knowledge which did not exist until they came—knowledge which later generations have used as if it had existed always. Some few of these men have combined with their scientific skill and intellectual capacity an unusual ability to affect the lives of the people with whom they worked. Dr. Curtis was one of these. Hundreds of men and women have taken into their own lives bits of the way their Professor Curtis looked upon life and carried on his work. This is a great and lasting tribute to any man."—Lewis Knudson and D. G. Clark.

PLANT PHYSIOLOGY

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EFFECT OF CERTAIN GROWTH REGULATORS ON SEED STALK DEVELOPMENT IN LETTUCE AND CELERY[1]

B. E. Clark and S. H. Wittwer

(WITH FIVE FIGURES)

Received February 4, 1949

Introduction

In many vegetables, the development of seed stalks destroys the value of market crops, yet it is essential for the production of seeds from which these crops are grown. In lettuce and celery, seed stalk formation, commonly called bolting, is very difficult to control because of unfavorable temperatures in many producing areas. The commonly employed attempts toward control are those of using varieties resistant to bolting, but sometimes inferior in quality, and of manipulating planting dates and other cultural practices.

A recently published symposium by Murneek and Whyte *et al.* (**19**) and a book by Whyte (**31**) provide comprehensive reviews of the effect of environment on plant development. Specific factors affecting seed stalk development in lettuce and celery have been discussed by Thompson and Knott (**25**) and by Bremer (**1**). Those factors affecting seed stalk initiation in celery have been studied by Thompson (**24**) and by Starring (**20**).

Obviously, interest in seed stalk formation goes beyond the field of crop production and extends into the realm of the fundamental study of plant development. In the latter field, plant physiologists have studied extensively environmental factors affecting the transformation from the vegetative to the reproductive stage of development and much has been learned concerning the influence of these factors. Little is yet known, however, about the biochemical mechanism involved. Nevertheless, recent discoveries indicate that, in some plants at least, certain already identified chemicals having growth-promoting properties may play a role in the physiological complex responsible for the initiation of reproduction.

A hastening of flowering in the pineapple through the application of synthetic growth regulators has been reported by Van Overbeek (**27, 28**), Clark and Kerns (**5**), and Cooper (**6**). A similar hastening of flowering

[1] Journal article No. 1017 (n.s.) of the Michigan Agricultural Experiment Station.

in other plants was observed by HITCHCOCK and ZIMMERMAN (**13**), THIMANN and LANE (**23**), STIER and DUBUY (**21**) and TANG and LOO (**22**).

The inhibition or retardation of flowering as a result of growth regulator applications has been reported by DOSTAL and HOSEK (**9**), JOHNSON (**16**), THURLOW and BONNER (**26**), GREEN and FULLER (**11**), WITTWER, COULTER and CAROLUS (**32**) and COULTER (**7**).

In experiments conducted by MURNEEK (**18**) and by HAMNER and BONNER (**12**) synthetic growth regulators failed to affect the date of flowering of treated plants. GALSTON (**10**), however, found that, although triiodobenzoic acid failed to hasten flowering, it increased the number of blossoms on plants which flowered at the usual time. ZIMMERMAN and HITCHCOCK (**33**) observed that triiodobenzoic acid caused normally vegetative axillary shoots in tomatoes to produce flowers instead of leaves.

These results obtained by applying growth-regulating substances to various crop plants suggested the possibility of influencing seed stalk development in lettuce and celery through the use of synthetic growth substances.

General methods

The experiments were carried out both in the greenhouse and in the field. In the greenhouse night temperatures ranged from 60–70 degrees Fahrenheit except during the early fall and late spring when warmer weather prevailed. In the field, usual commercial methods were followed in every respect possible.

The growth substances used in these experiments were 2,4-dichlorophenoxyacetic acid, alpha naphthaleneacetic acid, alpha ortho chlorophenoxypropionic acid, para chlorophenoxyacetic acid, and 2,3,5-triiodobenzoic acid, hereafter designated as 2,4-D, NAA, CLPP, CLPA, and TIBA respectively. The prepared solutions were sprayed onto the plants with small hand sprayers. Each plant was wet thoroughly and a special effort was made to reach the growing point. Responses of the plants to the growth substances were determined by weekly measurements of seed stalk lengths and by observation of the first appearance of blossom buds and of first open blossoms.

Statistical significance of the experimental results was determined either by the analysis of variance or by Student's "t" test, depending on which was considered the more appropriate for the data in question.

Lettuce experiments

EXPERIMENT 1

Preliminary greenhouse experiments indicated that the application of growth-regulating substances to winter crops of lettuce accelerated the rate of seed stalk elongation and that this acceleration was greatest when applications were repeated at regular intervals.

This experiment was set up to discover the effect of repeated applica-

tions of growth regulators on a spring crop of greenhouse lettuce. It was also designed to determine whether the effects of growth substances on seed stalk development are confined to the treated plants or whether they are transmitted to the untreated progeny of these plants.

METHODS.—Plants of the Grand Rapids variety were grown from seeds planted in the greenhouse on February 3, 1947. Seedlings were "pricked-off" on February 15 and transplanted to a greenhouse bed March 30. On April 5, individual plots of these plants were treated with NAA, CLPP, CLPA and TIBA, and 2,4-D respectively. Each treatment was repeated five times at weekly intervals.

Seeds produced by the treated plants were harvested in July and were stored in the laboratory until September. On September 8, 1947, seeds from plants of each treatment were planted in a greenhouse flat. On September 22 the resulting seedlings were transplanted to a ground bed in the greenhouse. This experiment with the progeny of treated plants was repeated in the spring of 1948. The design of the second trial was the same as the first except that there were three replications in the second trial whereas there were only two in the first.

RESULTS.—An analysis of the seed stalk length of the treated plants showed that, during the first three weeks of elongation, plants treated with 2,4-D and TIBA had seed stalks significantly longer than those of the control plants. During the succeeding two weeks of seed stalk elongation, however, there were no significant differences in seed stalk length among the plants of the several treatments.

The weekly increments in seed stalk lengths suggest the reason for the loss of significance as stalk elongation progressed. These weekly increments, as shown in table I, were greater during the early stages of

TABLE I

THE EFFECT OF GROWTH-REGULATING SUBSTANCES ON THE WEEKLY INCREMENT IN LENGTH OF SEED STALKS IN GREENHOUSE LETTUCE

TREATMENT	INCREMENT IN SEED STALK LENGTH (INCHES)			
	MAY 10–17	MAY 17–24	MAY 24–31	MAY 31–JUNE 7
Control	1.9	6.1	6.3	14.0
10 p.p.m. NAA	1.9	5.8	6.3	15.0
25 p.p.m. CLPP	3.1	7.7	6.9	13.7
5 p.p.m. CLPA	2.8	7.8	8.7	14.9
50 p.p.m. TIBA	3.9	8.6	7.8	14.3
5 p.p.m. 2,4-D	4.7	10.6	10.4	14.7

elongation for the treated plants than for those not treated. During the final week of observation, on the other hand, growth increments were essentially the same for all plants regardless of treatment. It is apparent, then, that changes in the seed stalk development of lettuce induced by

growth substances are most evident in the beginning of elongation and become negligible during the latter stages.

The progeny from the plants of the several treatments did not differ in their vegetative characteristics. Differences, however, did become apparent during the process of seed stalk elongation. This is clearly indicated by the data presented in table II. It is evident that seed stalk

TABLE II

THE EFFECT OF GROWTH-REGULATING SUBSTANCES ON THE LENGTH OF SEED STALKS IN THE PROGENY OF TREATED LETTUCE PLANTS

TREATMENT OF PARENT PLANT	LENGTH OF SEED STALK OF PROGENY (INCHES)							
	FIRST TRIAL				SECOND TRIAL			
	Nov. 22	Nov. 29	Dec. 6	Dec. 13	May 26	June 2	June 9	June 16
Control	10.9	14.1	19.5	29.8	8.3	14.9	27.3	27.3
NAA	12.1	17.8	23.8	32.6	10.0	18.9	31.7	41.8
CLPP	12.9	18.3	26.5	35.9	10.9	20.5	32.9	43.5
CLPA	13.4	19.7	25.7	35.7	12.7	22.9	35.2	46.5
TIBA	11.5	15.9	22.2	32.1	10.3	19.5	32.1	42.9
2,4-D	11.8	16.2	25.0	35.2	10.8	20.4	33.1	43.3
LEAST SIGNIF. DIFFERENCES 5% level	2.47	5.04	6.55	8.10	1.23	3.48	3.97	5.31

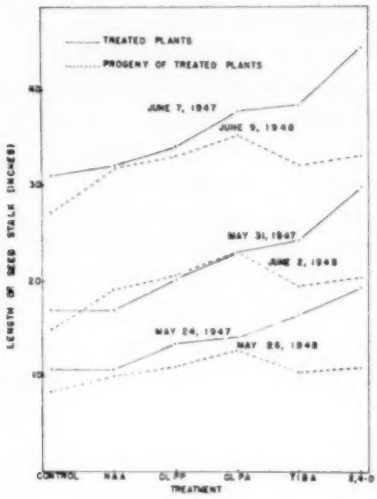

FIG. 1. Relative responses of treated lettuce plants and their progeny to growth regulating substances.

elongation was more rapid in the progeny of plants that had been treated with growth substances than it was in the progeny of plants that had not been treated.

The comparative responses of treated plants and their progeny to growth-regulating substances are illustrated in figure 1. The substances NAA, CLPP, and CLPA had about the same effect on the progeny as on the treated plants themselves. The effects of TIBA and 2,4-D, on the other hand, were much diminished in the second generation.

Experiment 2

Methods.—Seeds of two varieties of lettuce for field studies were planted in flats in the greenhouse on April 1, 1947, and transplanted to the field in four replicated blocks on May 1–3. At the proper time, individual plots were treated with water, 10 p.p.m. 2,4-D, 200 p.p.m. TIBA, or 50 p.p.m. CLPP. These chemicals were applied both in single applications and in three applications repeated at two-week intervals. Both the single and repeated applications were made at two different plant ages. The single applications were made when the plants were nine and 11 weeks old. The first set of repeated applications was made when the plants were 9, 11, and 13 weeks old and the second set, when they were 11, 13, and 15 weeks old. All of these treatments were applied in a factorial

TABLE III

The effect of growth substances on seed stalk development in field grown lettuce

Growth substance	Days from transplanting to appearance of blossom buds		Days from appearance of blossom buds to open blossoms		Length of seed stalk (inches)					
					Grand Rapids			Cornell 456		
	Grand Rapids	Cornell 456	Grand Rapids	Cornell 456	July 16	July 23	July 30	Aug. 8	Aug. 13	Aug. 18
Water	79	95	24	20	8.9	15.0	24.5	16.7	23.5	30.2
2,4-D	75*	94	24	20	14.1*	21.0*	28.8*	18.7	25.7	32.3*
TIBA	79	95	22	20	9.9	15.0	23.9	14.3*	21.9	28.6
CLPP	79	95	22	21	10.5	15.9	25.0	15.6	22.3	29.2

* Significant variation from the water (control) treatments. Student's "t" test.

arrangement making 16 treatments in all. The effects of the treatments were determined not only by periodical measurement of seed stalks but also by observation of the dates on which blossom buds and open flowers appeared.

Results.—Table III shows the effects of growth substances on the seed stalk development of these field-grown lettuce plants. In the Grand Rapids variety, the 2,4-D treatments produced significant variations, as compared to the control, both in length of seed stalk and date of blossom

bud appearance. They did not, however, cause a significant variation in the length of the interval between the appearance of blossom buds and the opening of blossoms. Neither the TIBA nor the CLPP treatments had any significant effect on the seed stalk development of the Grand Rapids variety.

In the Cornell 456 variety, none of the treatments produced significant variations in the dates on which blossom buds and open blossoms appeared. The only significant variations from the controls in length of seed stalk occurred on August 8 in the TIBA treated plants and on August 18 in the 2,4-D treated plants. Seed stalks of the former plants were significantly shorter than the controls, whereas those of the latter were significantly longer.

Table IV shows the effects of the time and method of application on the

TABLE IV

EFFECT OF TIME AND METHOD OF APPLICATION ON RESPONSE OF FIELD GROWN GRAND RAPIDS LETTUCE TO 10 P.P.M. OF 2,4-D

METHOD OF APPLICATION	DAYS FROM TRANSPLANTING TO APPEARANCE OF BLOSSOM BUDS	DAYS FROM APPEARANCE OF BLOSSOM BUDS TO OPEN BLOSSOMS	LENGTH OF SEED STALK (INCHES)		
			JULY 16	JULY 23	JULY 30
1 application, 9 weeks old	76	22	11.9	19.4	27.6
1 application, 11 weeks old	76	25	13.7	19.6	27.3
3 applications, 9, 11, and 13 weeks old	72	25	17.4	24.7	31.6
3 applications, 11, 13, and 15 weeks old	76	24	13.5	20.3	28.5

responses of plants to 2,4-D. Although none of the variations occurring in table IV are statistically significant, there is some indication that the repeated treatments applied when the plants were 9, 11, and 13 weeks old were more effective in hastening seed stalk development than were the other treatments.

EXPERIMENT 3

METHODS.—Although COULTER (**7**) and WITTWER, COULTER and CAROLUS (**32**) found that CLPP retarded seed stalk development in celery, no instance of significant retardation by CLPP was observed in the lettuce experiments described above. In fact, CLPP tended to hasten seed stalk development. Since Coulter's results were obtained by treating young celery plants before induction had occurred, it was thought that seed stalk development in lettuce might be retarded rather than stimulated if the plants were treated at a relatively young age. It was thought, also, that concentrations of CLPP greater than those already used might have a retarding, rather than an accelerating, effect on seed stalk development.

To test these possibilities, seeds of the Grand Rapids variety were planted in the greenhouse September 17, 1947. Later they were transplanted to plots containing five plants each, and on October 17, individual

TABLE V

EFFECT OF VARIOUS CONCENTRATIONS OF CLPP ON THE TIME REQUIRED FOR THE APPEARANCE OF BLOSSOM BUDS AND OPEN BLOSSOMS ON LETTUCE PLANTS

CONCENTRATION CLPP, P.P.M.	DAYS FROM SEEDING TO TREATMENT	DAYS FROM TREATMENT TO APPEARANCE OF BLOSSOM BUDS	DAYS FROM APPEARANCE OF BLOSSOM BUDS TO OPEN BLOSSOMS
0	30	82	31
5	30	85	33
25	30	86	32
50	30	85	31
75	30	87*	33
100	30	86	33
200	30	85	33
300	30	89*	32
400	30	88*	33

* Significant variation from control as determined by analysis of variance.

plots were treated with eight different concentrations of CLPP including 5, 25, 50, 75, 100, 200, 300 and 400 p.p.m. Records of the rates of seed stalk elongation and the dates of the first appearance of blossom buds and of first open blossoms are recorded in table V and figure 2.

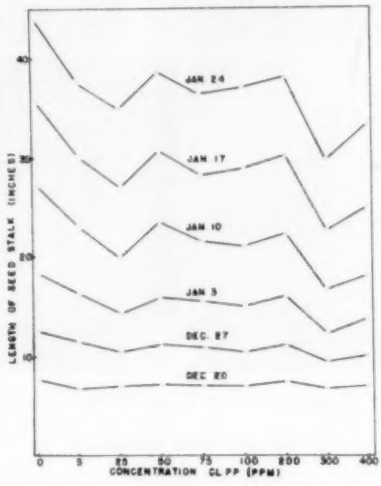

FIG. 2. Effect on subsequent seed stalk elongation of applying various concentrations of CLPP to month-old lettuce plants.

RESULTS.—Treatments of 75, 300 and 400 p.p.m. of CLPP significantly delayed the appearance of blossom buds. None of the treatments, how-

ever, had a significant effect on the length of the interval between the appearance of blossom buds and the appearance of open blossoms.

The effect of the CLPP treatments on the elongation of seed stalks of the treated plants is portrayed in figure 3. The points on the graph in-

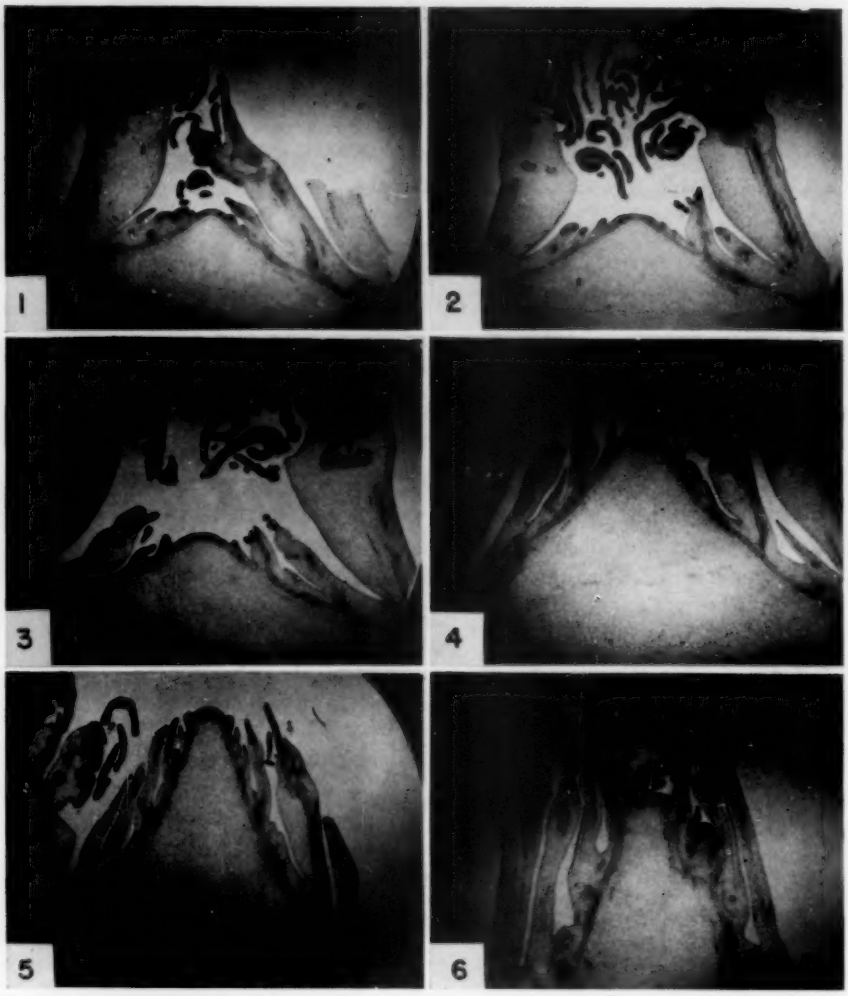

FIG. 3. Stages in the development of terminal growing points of celery stems.

dicate the average heights of the seed stalks on the dates specified. The vertical distances between points indicate increments in length of seed stalk.

On December 20, none of the treatments varied significantly from the control. On subsequent dates, plants treated with the concentrations given below had seed stalks significantly shorter than the control:

December 27—25, 75, 100, 300, and 400 p.p.m.
January 3—all concentrations except 5 p.p.m.

January 10—all concentrations
January 17—all concentrations
January 24—all concentrations

During the weeks indicated, plants receiving the following concentrations of CLPP showed significantly smaller increments in seed stalk length than did the controls.

December 20–27; 25, 100, 300 and 400 p.p.m.
December 27–January 3; 5, 25, 75, 100, 300 and 400 p.p.m.
January 3–10; 25, 75, 100, 200, 300 and 400 p.p.m.
January 10–17; 5, 25, 75, 300 and 400 p.p.m.
January 17–24; none.

Two interesting points are brought out by figure 2 and the data above; first, that there were no significant differences in seed stalk length on the first date of measurement, December 20, and second, that, during the last week of the experiment, January 17–24, there were no significant differences in the increments in seed stalk length. It is apparent, then, that all of the significant responses to the various concentrations of CLPP took place between December 20 and January 17, the period during which the seed stalks elongated from approximately seven to 30 inches.

Celery experiment

Object of study

The lettuce experiments already described showed that, under certain conditions at least, growth-regulating substances are capable of influencing the rate of seed stalk development. They did not indicate, however, whether growth substances influenced only the rate of seed stalk elongation, or whether they also affected the date of the differentiation of floral organs. Neither did these experiments give any indication of the role in seed stalk development of the growth substances normally occurring in plants.

In order to gain information on the latter subjects, an experiment was set up to determine the effects of variety, induction treatment, and the application of growth regulators on the morphological differentiation, phytohormone content, and elongation of celery stems. Celery, rather than lettuce, was used in this experiment because in celery, it is easier experimentally to control environmental factors causing seed stalk initiation.

General procedure

Two varieties of celery were selected for this study, Cornell 19, which bolts quite readily, and Tall Strain, Non-Bolting Golden Plume, a variety highly resistant to bolting. Seeds of the two varieties were planted in No. 2 vermiculite on February 9, 1948 in a 60–70 degree greenhouse. On March 12 to 17, the resulting seedlings were transplanted to flats of muck soil with a spacing of two by two inches between seedlings.

On April 13, plants from each of the two varieties were separated into three groups for treatment with growth substances. Those of the first group were treated with 50 p.p.m. 2,4-D, those of the second, with 100 p.p.m. CLPP, and the plants of the third received no treatment.

Two days after the celery plants had been treated, half of the plants of each treatment were moved to a cold frame where they were exposed to night temperatures ranging from 34 to 55° F until moved to the field on May 19. The other plants were left in the greenhouse until they were similarly transferred to the field on the same date.

The treatments thus applied to the celery plants are summarized below:

A.	Cornell 19	No growth substance	Greenhouse
B.	Cornell 19	No growth substance	Cold frame
C.	Cornell 19	50 p.p.m. 2,4-D	Greenhouse
D.	Cornell 19	50 p.p.m. 2,4-D	Cold frame
E.	Cornell 19	100 p.p.m. CLPP	Greenhouse
F.	Cornell 19	100 p.p.m. CLPP	Cold frame
G.	Golden Plume	No growth substance	Greenhouse
H.	Golden Plume	No growth substance	Cold frame
I.	Golden Plume	50 p.p.m. 2,4-D	Greenhouse
J.	Golden Plume	50 p.p.m. 2,4-D	Cold frame
K.	Golden Plume	100 p.p.m. CLPP	Greenhouse
L.	Golden Plume	100 p.p.m. CLPP	Cold frame

In the field, the plants were set into plots of muck soil May 22–26. Plants of each treatment were set into two areas. The first contained five blocks, each of which was composed of 12 randomized plots corresponding to the 12 treatments described above. Each plot in this area consisted of a row 33 feet long and containing 66 plants. The plants used for morphological observations and phytohormone determinations were taken from this area.

The second area of plants was arranged in the same manner as the first with the exception that each plot consisted of a row seven feet long containing 10 plants. These plants were allowed to produce seed stalks which were measured at weekly intervals.

Morphological observations

Methods.—On April 27 and each week thereafter until July 27, five celery plants for morphological observations were taken from each of the treatments described above. Each of these plants was taken from a different flat while the plants were in the greenhouse and cold frame and from a different plot after the plants had been moved to the field. After the leaves and roots had been removed from the selected plants, excess tissue was trimmed from the area around the growing points. The stem apices thus

trimmed, were killed and fixed in F.A.A. killing and fixing solution. The tissues were then dehydrated by the tertiary butyl alcohol method described by JOHANSEN (**15**). The dehydrated tissues were embedded in paraffin and sections 14 microns thick were cut from them on a rotary microtone. The cut sections were stained with Delafield's Haematoxylin and mounted in balsam. Slides thus prepared were observed under a microscope and a record was kept of the date of the first ascertainable seed stalk differentiation in plants from each treatment.

RESULTS.—Stages in the development of terminal growing points of celery stems are shown in figure 3. The first three stages show a broadening of the growing point. This broadening was not associated with subsequent seed stalk development. It occurred in all of the plants regardless of whether floral primordia and seed stalk elongation subsequently followed. Stages 4, 5, and 6, on the other hand, were associated with seed stalk development. None of these occurred in any plants which did not subsequently produce seed stalks. Stage 4 shows the first visible indication of elongation. In this stage, the internodes in the region of the growing point have elongated slightly to produce a cone-shaped tip. Stage 5 is characterized by a still greater elongation in the vicinity of the growing point and by the appearance of primordial axillary shoots. The primordia of inflorescences are apparent in stage 6.

The growing points of the plants of all treatments remained in stage 1 from the time of the first collection of samples on April 27 through the collection on June 8. On June 15, all of the plants had terminal growing points which had reached stage 2, and on June 22, all growing points had reached stage 3. Only treatments B, D, and F (Cornell 19 plants which had received an induction treatment in the cold frame) produced plants whose terminal growing points advanced beyond stage 3. The growing points of all other plants remained in stage 3 until the end of the experiment on July 27.

TABLE VI

THE EFFECT OF GROWTH SUBSTANCES ON THE DIFFERENTIATION OF PRIMORDIAL UMBELS IN THE TERMINAL GROWING POINTS OF CELERY STEMS

TREATMENT	NUMBER OF GROWING POINTS CONTAINING PRIMORDIAL UMBELS				
	JUNE 29	JULY 6	JULY 13	JULY 20	JULY 27
B Control	0	1	3	4	5
D 2-4-D	0	1	1	1	5
F CLPP	0	0	2	4	5

Plants in the three treatments which produced seed stalks had terminal growing points in stage 4 of figure 3 approximately June 29. Their growing points had progressed to stage 5 about July 6, and stage 6 about July 13. Beyond stage 3, however, the development of growing points of different

plants proceeded at different rates so that not all of the plants of a single treatment were in the same stage at a given date.

Table VI shows the effect of growth substances on the appearance of primordial umbels in plants of treatments B, D, and F. None of the plants possessed primordial umbels on June 29. On July 27, on the other hand, all five plants collected from each treatment contained primordia. Between these two dates, the rate of differentiation of primordia appeared to be more rapid in control plants and those treated with CLPP than in plants treated with 2,4-D. These results, however, could hardly be considered conclusive.

Phytohormone* determinations

Methods.—On April 27, and every week thereafter until July 27, 25 plants were taken from each of the treatments listed above for phytohormone extractions. The plants included in the samples were removed in proportional numbers from each flat while the plants were in the greenhouse and cold frame, and from each plot after the plants had been moved to the field in the same manner as material used in morphological observations.

Upon removal from the soil, the plants were severed at the junctures of their stems and roots. After this, the leaves were pulled from the stem plate and discarded. These operations produced pieces of stem tissue free from roots and leaves. While the plants were young, the pieces of tissue were sliced and prepared for extraction without further treatment. In older plants, a cylinder of tissue about 5 mm. in diameter and 4 mm. high was cut from the stem tissue surrounding the apical meristem. This was then sliced and prepared for extraction. In both the young and old plants, the sliced stem tissue was frozen immediately with dry ice. The procedure outlined provided three to five grams of frozen tissue from each of the 12 treatments. This frozen tissue was taken to the laboratory where three-gram portions were weighed out from each lot of tissues and placed in Erlenmeyer flasks. The tissue in each flask was then covered with 20 ml. of freshly distilled, peroxide-free ether. At the end of six hours, this ether was decanted and replaced with another 20 ml. portion. At the end of another 15 hours, the second portion of ether was decanted and replaced with a third. The third portion was decanted at the end of another 22 hours. The first two portions of ether were refrigerated until the third portion was decanted. Then all three portions were placed together in an evaporation dish and to them was added another 5 ml. portion of ether used in rinsing the tissue in the Erlenmeyer flasks.

The ether in the evaporating dish was evaporated before a fan until only an aqueous residue remained. This residue was further evaporated until

* The term phytohormone as used here refers to substances capable of causing curvature of cucumber hypocotyls. It is not known whether these are the same substances as those causing curvature of the Avena coleoptile and commonly called auxins.

less than 2 ml. remained in each evaporating dish. Each residue was then brought to a volume of 2 ml. to produce the solution to be assayed for phytohormone content.

The phytohormone determinations were made by means of a modification of the cucumber injection method described by KRIBBEN (**17**). This method was used primarily because facilities for the standard Avena test were not available. There was, however, another reason for using this test. Experiments with selective herbicides have shown that some growth sub-

FIG. 4. Method of injecting phytohormone extracts into cucumber cotyledons.

stances produce an effect on broad leaf plants different from their effect on the grasses. Therefore, it might be expected that a dicotyledonous plant like the cucumber would give a better indication of the phytohormone content of celery growing points than would the Avena coleoptile.

The cucumber test as described by Kribben was carried out on a revolving table in the greenhouse. It was found in tests preliminary to this experiment, however, that the accuracy of the test could be improved by placing the plants in a high humidity incubator previous to testing. If the plants were not placed in a saturated atmosphere previous to injection, curvature was produced even by injections of pure water. This apparently resulted from unilateral hydration of the hypocotyl and may account for some of the erratic results obtained in the early use of this test.

To supply seedlings for the cucumber tests, seeds of the National Pickling variety were planted in No. 2 vermiculite. As soon as the seedlings had emerged and their cotyledons had separated, they were transplanted to trays of soil in the greenhouse. The soil trays were three inches wide, three inches deep, and 17 inches long. Ten seedlings were transplanted into a single row in each tray in such a way that their cotyledons were arranged

crosswise of the long axis of the tray as indicated in figures 4 and 5. About four days after transplanting, as soon as the first true leaf was visible, the seedlings were moved to an incubator in a dark room. The incubator was kept at 30° C and its air was kept saturated with moisture by placing open containers of water on its shelves. The trays of cucumbers were also well watered before being placed in the incubator. After the cucumber seedlings had been in the incubator for 24 hours, the trays were removed one by one, and the extracts to be tested were injected into the seed-

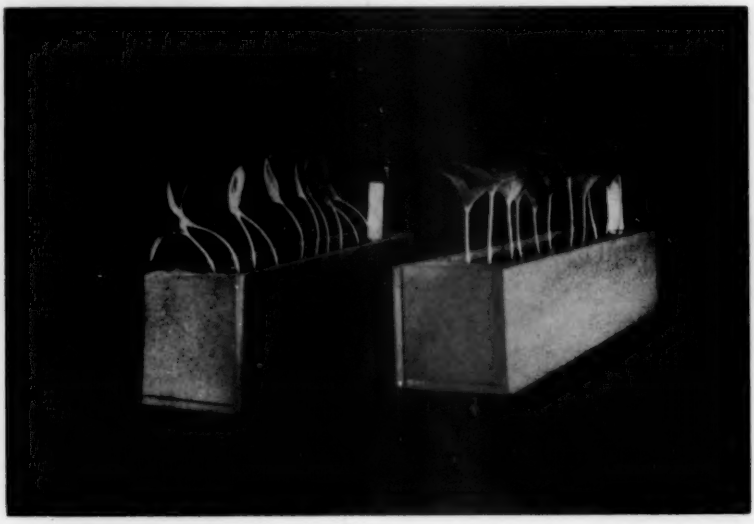

FIG. 5. Responses of cucumber seedlings to hypodermic injections. Left—1 p.p.m. indoleacetic acid. Right—water.

ling cotyledons. All of these operations were carried out with the aid of a red "safe" light in a dark room so that there would be no phototropic curvature of the hypocotyls.

The method followed in injecting solutions into cucumber cotyledons is illustrated in figure 4. At about the center of each cotyledon there are two veins which branch from the midrib. The hypodermic needle was inserted between the upper and lower epidermis of the cotyledon in the axil of one of these veins. After the needle had been inserted, .05 ml. of the extract solution was injected into the cotyledon. A small droplet of solution was usually forced out around the needle, but this droplet was later reabsorbed. The injections were made with a tuberculin syringe graduated in hundredths of a milliliter and equipped with a 27 gauge needle one-half inch long.

In making the phytohormone tests, extract solutions were injected into one cotyledon of each seedling. The extract from the plants of each treatment was injected into one tray of ten seedlings. Thus, 12 trays of seedlings received injections of extracts each week. In addition to the extract injections, one tray of seedlings was injected with water and another with

a 1 p.p.m. solution of indoleacetic acid. These two treatments served as controls. Figure 5 shows trays of seedlings having received these two treatments.

After treatment, the cucumber seedlings were returned to the incubator for a period of two hours. At the end of the two-hour period, they were removed from the incubator and the curvature of the hypocotyls was measured. In order to measure this curvature, the hypocotyl was severed just below the cotyledons and again at the surface of the soil. The lower end of the hypocotyl was placed along a vertical line on a piece of paper. A line was then traced along the side of the upper end of the hypocotyl where it departed from the vertical line. Later, this line was extended and the angle of divergence from the vertical line was measured with a protractor. This angle of divergence represented the curvature of the hypocotyl produced by the injected extract plus curvature occurring naturally in the growth of the seedlings. The curvature produced by a given extract, therefore, was obtained by subtracting the degrees of curvature of the control (water injected) seedlings from the degrees of curvature of the seedlings injected with the extract in question.

RESULTS.—The phytohormone determinations indicated that, in respect to phytohormone activity, there were three critical periods in the development of the terminal growing points of celery stems. In this experiment, the first critical period began about three weeks after the plants had been exposed to induction temperatures in the cold frame and extended over a period of four weeks (May 4–25). The second period (June 15) occurred approximately two weeks previous to the elongation of seed stalks and differentiation of umbels. The period of initial seed stalk elongation and differentiation of flower primordia (July 6–13) constituted the third critical period. At times other than those indicated, the phytohormone activity of the celery stem extracts was so low that it could not be measured accurately by the methods employed.

The results of phytohormone determinations made during the critical periods described above are presented in table VII. The negative values in table VII represent curvatures of cucumber hypocotyls toward the cotyledons receiving the injections (positive curvature). Other values represent curvatures away from the injected cotyledons (negative curvature). Curvatures toward the injected cotyledons were the result of inhibition of growth by the injected extracts. Curvatures away from the injected cotyledons were caused by stimulation of growth. Extracts causing inhibition were encountered only on June 15 and even these extracts, upon dilution, were found to stimulate growth. It appears, therefrom, that the growth inhibition produced by these extracts was merely an expression of their concentrations. On all dates other than June 15, the curvature of cucumber hypocotyls produced by extracts of celery stem apices was less than that produced by the standard solution of 1 p.p.m. indoleacetic acid.

It can be seen from table VII that, on May 11, 18, and 25, the apical stem tissue of plants in the cold frame had a significantly higher phytohormone content than that of plants in the greenhouse, as indicated by the assay methods employed. Also, on May 4 and 11, stem tissues from

TABLE VII

EFFECT OF EXPERIMENTAL FACTORS ON THE PHYTOHORMONE CONTENT OF THE MERISTEM REGION OF CELERY STEMS

Experimental factor	Degrees curvature of cucumber hypocotyls						
	May 4	May 11	May 18	May 25	June 15	July 6	July 13
Induction Treat.							
Cold frame	13.7	21.8	23.7	14.8	12.1	10.1	10.4
Greenhouse	16.2	14.4	10.2	5.9	−3.3	4.6	13.4
Differences	2.5	7.4*	13.5*	8.9*	.....	5.5	3.0
Variety							
Cornell 19	22.3	21.0	18.0	12.4	15.6	10.5	18.0
Golden Plume	7.6	13.5	15.9	8.3	−6.8	4.2	5.8
Differences	14.7*	7.5*	2.1	4.1	.....	6.3*	12.2*
Growth Substance							
Control	8.5	10.6	10.6	7.1	−3.1	5.9	7.8
2,4-D	13.7	10.6	9.7	8.2	10.0	5.0	7.9
CLPP	7.7	15.0	13.6	5.4	2.8	3.8	8.1
Differences							
Control—2,4-D	5.2	0.9	0.9	1.1	.....	0.9	0.1
Control—CLPP	0.8	4.4	3.0	1.7	.....	2.1	0.3
Stage of Development	Induction				Pre-differentiation	Differentiation	

* Significant difference. Student's "t" test.

plants of the Cornell 19 variety were higher in phytohormone content than those from plants of the Golden Plume variety. It is apparent from the data presented that significant differences resulting from the induction treatment occurred later than those associated with variety. Cornell 19 responded to the cold treatment more rapidly than Golden Plume. Therefore, there were significant differences between the two varieties relatively early in the induction period. Later, after both varieties had responded to the cold treatment, the difference between varieties decreased, but the difference between cold and warm temperature treatments increased.

On June 15 the situation was the reverse of that occurring during the induction period. At this time, the phytohormone content of the apical stem tissue of Cornell 19 plants was lower than that of the Golden Plume plants. Also, the stem tissue of plants which had received a cold exposure was lower in phytohormone content than that of plants which had received

no cold exposure. It was impossible, however, to determine the statistical significance of differences observed on June 15 because the negative readings recorded were not true mathematical representations of the phytohormone activity of the extracts involved. On July 6–13, the stem tissue of Cornell 19 plants was significantly higher in phytohormone content than that of Golden Plume plants. The stem tissue of the cold-induced plants also averaged somewhat higher than that of plants which had received no induction treatment.

Although variety and induction treatment sigficantly affected the phytohormone content of celery stem tissues, the application of growth substances did not produce significant effects at any time during the progress of this experiment.

Bolting observations

Methods.—Plants in the field area set aside for bolting observations were observed for seed stalk elongation. Seed stalks became apparent on July 23, and their heights were measured at weekly intervals thereafter until August 27. The measurements were taken from the surface of the soil to the highest point on the seed stalk.

Results.—Treatments B, D, and F were the only treatments which resulted in seed stalk elongation. These three treatments were the ones containing thermally induced plants of the Cornell 19 variety. At no time during the period of measurement, were the seed stalks of plants in treatment F (CLPP treatment) significantly different in height from those of plants in treatment B (controls). On the first two dates of measurement (July 23 and 30), the plants of treatment D (2,4-D treatment) had seed stalks

TABLE VIII

The effect of growth-regulating substances on the weekly increment in the elongation of celery seed stalks

Period of growth	Increment in seed stalk length (inches)		
	Control	CLPP	2,4-D
July 23–30	5.3	5.5	3.5
July 30–Aug. 6	4.9	5.1	3.9
Aug. 6–13	6.4	6.8	5.6
Aug. 13–20	5.3	5.5	5.3
Aug. 20–27	7.3	8.0	7.5

which were significantly shorter than those of the control plants. On succeeding dates, however, these did not vary significantly from the controls. The reason for significant differences during the early stages of elongation and lack of significance in later stages is suggested by the data in table VIII showing the weekly increments in length of seed stalk. During the first two weeks of elongation, the increments for the 2,4-D treated plants were less than those for the controls and for the CLPP treated plants. During

the last two weeks of measurement, on the other hand, the growth increments were essentially the same for plants of all three treatments.

Discussion

A feature of fundamental interest observed in these investigations was the transmission of the effects of growth substances from one generation to the next. That the effects of growth substances on seed stalk elongation can be transmitted from a treated parent to its nontreated progeny was clearly demonstrated. Just how this transmission was accomplished, however, awaits further study.

The effect of synthetic growth substances on the progeny of treated plants was reported previously by HITCHCOCK and ZIMMERMAN (**14**). They observed that dandelion plants which had been treated with 2,4,6-trichlorophenoxyacetic acid produced progeny which were markedly delayed in their flowering. Some treatments applied by Hitchcock and Zimmerman, however, resulted in the production of seeds of reduced viability. Seeds produced by the treated lettuce plants described above showed no loss of vitality and the seedlings produced by them displayed no visible formative effects

Another important feature observed in the application of synthetic growth substances to lettuce and celery plants was the variability in the results obtained. In several experiments, for instance, the rate of seed stalk elongation in lettuce tended to be accelerated by application of CLPP to two-month-old plants. In an experiment where CLPP was applied to one-month-old plants, on the other hand, the rate of seed stalk elongation was retarded. Furthermore, this retarding effect did not appear to be a function of concentration as it was in pineapple experiments reported by CLARK and KERNS (**5**).

In celery, WITTWER, COULTER, and CAROLUS (**32**) and COULTER (**7**) observed that seed stalk elongation was completely inhibited during the 1947 season by the application of 100 p.p.m. CLPP and that it was accelerated by 50 p.p.m. 2,4-D. The results presented above, on the other hand, show that, during the 1948 season, 100 p.p.m. CLPP had no effect on seed stalk elongation and 50 p.p.m. 2,4-D caused a slight retardation of elongation. These results were obtained in spite of the fact that the procedure followed in 1947 was duplicated as nearly as possible in 1948.

It is apparent that conditions not under experimental control influenced the results of these experiments. In lettuce, the age of the plant at the time of treatment appeared to be the factor determining its response to growth substances. There is little evidence that this is true in celery, however.

The results of the phytohormone assays of celery stem apices suggest the possibility that flowering may not be a response to the mere presence of a special flower-forming substance as proposed by CAILAHJAN (**2** and **3**) and supported by HAMNER and BONNER (**12**), but that it may be caused

by local concentrations of ordinary phytohormones such as those capable of causing cell enlargement (auxins). This latter theory was proposed by CHOLODNY (**4**) and is supported by the experiments of VAN OVERBEEK and CRUZADO (**29**) and CURTIS and CHANG (**8**). VAN OVERBEEK and his associates (**27, 28, 29, 30**) have carried this theory still further and suggest that flowering results from the release of bound auxin from leaf blades and the accumulation of the auxin thus released in the stem apices. The results herein reported seem to be explained better by this theory than by the florigen theory of Cailahjan.

Summary and conclusions

Lettuce and celery plants were treated with aqueous solutions of 2,4-dichlorophenoxyacetic acid (2,4-D), alpha-orthochlorophenoxypropionic acid (CLPP), 2,3,5-triiodobenzoic acid (TIBA), para-chlorophenoxyacetic acid (CLPA), and naphthaleneacetic acid (NAA).

In general, when any of these substances was applied to eight- to 12-week-old lettuce plants, seed stalk elongation was hastened, the effect being greater for repeated than for single applications. In contrast, however, in the one case in which CLPP was applied to month-old plants there was a significant retardation.

The effects of growth substances, whether retarding or stimulating, occurred during the early stages of seed stalk elongation and were associated with a corresponding effect on the date of flowering.

Alterations in seed stalk development induced by the applications of growth substances were transmitted to the progeny of the treated plants.

In an experiment conducted to determine the effect of variety, induction treatment, and growth regulators (2,4-D and CLPP) on the morphological development, phytohormone content, and elongation of celery stems, only thermally induced Cornell 19 plants produced seed stalks. Among these, elongation was slightly retarded by 50 p.p.m. 2,4-D and not affected by 100 p.p.m. CLPP.

On a comparative basis, those factors which caused seed stalk formation were associated with a high concentration of phytohormones in the stem apices during the induction period, a low concentration during the period just preceding flower differentiation, and a high concentration again at the time of the differentiation of primordial umbels.

Because of the variability in the responses of plants to the application of synthetic growth regulators, these substances cannot yet be recommended for the practical control of seed stalk development. Nevertheless, although no clear cut treatments for retarding or accelerating bolting have been indicated, several phases of the problem have been elucidated.

The authors are indebted to Drs. P. M. Harmer and J. F. Davis of the Department of Soil Science at Michigan State College for providing the muck soil in which the experimental celery plants were started and the

field plots into which they were transplanted. The authors are also indebted to Dr. H. M. Sell of the Department of Agricultural Chemistry for supplying the peroxide-free ether used in phytohormone extractions.

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INDOLEACETIC-NICOTINIC ACID INTERACTIONS IN THE ETIOLATED PEA PLANT

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(WITH THREE FIGURES)

Received May 2, 1949

The present author has previously reported that indoleacetic acid (IAA) and nicotinic acid (NA) may interact synergistically in the growth of pure cultures of excised asparagus stem tips (**4**) and under certain conditions in cultures of *Lactobacillus arabinosus* 17–5 (**7**). The present investigations describe further instances of interaction between these compounds, and provide additional data which may contribute to an understanding of the mechanism of the effect.

Because of previous experience with growth responses of etiolated peas (**5, 6**) and because of the known requirement of pea roots for nicotinic acid (**3**), it was considered desirable to utilize etiolated pea plants for the present investigation.

Materials and methods

Alaska peas (*Pisum sativum* L.) were soaked in tap water for two hours and were then sown in flats containing washed river sand. They were allowed to germinate and grow in a dark room whose temperature was 25° C. The only light to which they were exposed prior to harvest was phototropically inactive light transmitted through an orange-red Corning filter (#348).

At the age of seven to eight days after planting, the etiolated pea epicotyls were about 20 cm. tall, and had three nodes on the erect portion of the stem. The two lower nodes produce scales which do not normally expand into leaves. In the axils of these scales are found one or more buds which customarily remain dormant. For the experiments on bud growth herein described, the experimental material consisted of 10 cm. long sections of the epicotyl, containing scale node #2 in the median third of the section. Such epicotyl sections were placed erect in tap water for two hours immediately after cutting to deplete them of reserve materials. They were then placed in groups of 12 into 50 ml. Erlenmeyer flasks containing 5 or 10 ml. of the solution to be tested. The orifice of the flask was closed by the upper end of the epicotyl sections and by a small cotton plug. To avoid undue microbial contamination solutions were replaced every two to three days.

Flasks containing experimental epicotyl sections were either allowed to remain in the dark room or were removed to a light chamber kept at 26° C.

Light of about 500 foot candles intensity was supplied by batteries of 40 Watt "Daylight" and "White" Mazda fluorescent tubes mounted about 12 inches above the experimental shelf.

Experiments on root initiation were conducted by inverting groups of similar epicotyl sections into auxin-containing test solutions for 24 hours in the dark, and then reinverting them into auxin-free solutions containing 2% sucrose. After seven days, numerous roots were apparent at the bases of such epicotyls. These were counted to give a measure of the comparative root-initiating effect of the test solutions.

In order to avoid complications due to differential entry of NA at different pH values, nicotinamide (NAm) was used in place of the free acid. The 1(−)tryptophane and nicotinamide used were Merck products; the indoleacetic acid was the Eastman Kodak product, and the 1-kynurenine sulphate and 3-hydroxyanthranilic acid were obtained from Drs. H. K. Mitchell and J. F. Nye of these laboratories.

Assays for nicotinic acid were made by the SNELL and WRIGHT technique (**13**) utilizing *Lactobacillus arabinosus* 17–5 as an assay organism. Auxin assays were conducted by the conventional Avena technique of WENT and THIMANN (**18**).

IAA-NAm interactions in root initiation

WENT (**17**) has shown that IAA and other auxins can cause the initiation of numerous root primordia at the base of pea epicotyl sections. In such tests, it is general to apply the auxin apically, because of the basipetal polarity of transport of this compound. In the experiments outlined below, IAA and NAm were incorporated into the test solutions into which the epicotyls were inverted for 24 hours in the dark. The epicotyls were then removed from the solutions, rinsed free of auxin, and reinverted into auxin-free media containing the experimental concentration of NAm, 2% sucrose and M/15 pH 6.1 phosphate buffer. Duplicate flasks were prepared for this secondary treatment, one set being stored in the dark room, and the other being exposed to the light. The solution was replaced as needed, and the roots counted after the epicotyls had remained in the solution for an additional six days.

As seen from the data of table I, root initiation proceeds well in the dark, but is strongly inhibited by light. Traces of NAm cause the appearance of some roots; these are presumably the pre-existing root primordia of the epicotyls. The addition of auxin results in the formation of new root primordia, and if optimal NAm and IAA concentrations are combined, both the number and length of roots produced are sharply increased, the effect being much greater than a simple additive one. The maximum stimulatory effect of NAm is exerted at about 0.5 γ/ml.: concentrations in the neighborhood of 50 γ/ml. are distinctly inhibitory.

TABLE I

INTERACTIONS OF IAA AND NAm IN THE ROOTING OF ETIOLATED PEA EPICOTYLS. GROUPS OF 10 CM. LONG EPICOTYLS INVERTED INTO 10 ML. OF TEST SOLUTION CONTAINED IN 50 ML. ERLENMEYER FLASKS KEPT IN THE DARK. AFTER 24 HOURS, EPICOTYLS REINVERTED INTO IAA-FREE SOLUTIONS CONTAINING THE SAME NAm CONCENTRATION, 2% SUCROSE AND M/15 pH 6.1 PHOSPHATE BUFFER. ROOTS COUNTED AFTER END OF 7TH DAY OF TREATMENT. DARK = OCCASIONAL WEAK RED LIGHT OF THE AVENA ROOM; LIGHT = 500 F.C. MIXED "WHITE AND "DAYLIGHT" FLUORESCENT LIGHT.

TEST SOLUTION		NUMBER AND LENGTH OF ROOTS PRODUCED			
		DARK		LIGHT	
γ/ML. IAA	γ/ML. NAm	NO. OF ROOTS PER 10 EPICOTYLS	AV. LENGTH (MM.)	NO. OF ROOTS PER 10 EPICOTYLS	AV. LENGTH (MM.)
0	0	0	.....	0	.....
0	0.5	15	4.9	0	.....
0	5.0	0	.....	1	0.2
0	50	2	1.6	0	.....
10	0	18	11.0	8	6.4
10	0.5	53	31.8	0	.....
10	5.0	22	10.2	0	.....
10	50	18	9.1	0	.....

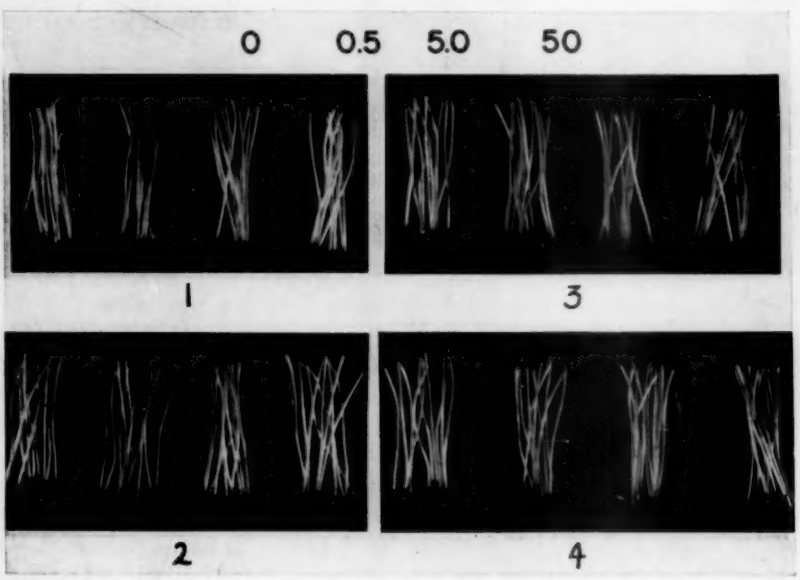

FIG. 1. Interaction of IAA and NAm in bud growth, rooting and apical shrinkage of etiolated pea epicotyls. Description in the text. In each series of four groups of epicotyls the concentrations of NAm were, from left to right, 0, 0.5, 5.0 and 50 γ/ml. 1. Dark O IAA. 2. Dark 10 γ/cc. IAA. 3. Light O IAA. 4. Light 10 γ/cc. IAA.

IAA-NAm interactions in bud growth

It was fortuitously noted that in rooting experiments performed in the light, those test solutions devoid of IAA but containing 50 γ/ml. NAm produced vigorous growth of the normally dormant axillary buds (cf. figure 1). This effect was not apparent if (a) auxin was supplied in the test solution or (b) light was omitted. These results imply that the normal IAA induced-lateral bud inhibition described by Thimann and Skoog (**15**) is operative, and also that other essential growth factors are synthesized only in the light. Data obtained by weighing or measuring the length of the buds produced are shown in table II.

TABLE II

IAA–NAm interactions in bud growth of etiolated peas. Procedural details as in table I. All experiments conducted in the light

Test solution		Fresh wt. (mg.) of buds per 10 epicotyls						Av. length of buds
		Expt. P-33*		Expt. P-37		Expt. P-38		Expt. P-67
γ/ml. IAA	γ/ml. NAm	Dark	Light	Dark	Light	Dark	Light	Light
0	0	25	25	21	29	16	58	3.1 ± 0.45
0	0.5	15	30	.....	.....	.....	.....	
0	5.0	10	25	.....	.....	.....	.....	
0	50	30	*180*	17	*78*	16	*119*	7.0 ± 1.4
10	0	15	15	.....	.....	11	18	
10	0.5	10	15	.....	.....	.....	.....	
10	5.0	15	15	.....	.....	.....	.....	
10	50	15	15	14	19	13	13	

* Weight to nearest 5 mg.

Since NAm obviously stimulates bud growth and IAA inhibits bud growth it was thought desirable to set up an experiment in which IAA and NAm levels were simultaneously varied over large ranges. In such a way a more quantitative picture of the nature of the interaction could be obtained.

Typical data are presented graphically in figure 2. It is evident from this experiment that at any IAA level, bud growth is most stimulated by 25 γ/ml. NAm, but the range over which NAm may exert its effect is more or less limited by the IAA level. The implications of such data will be discussed later in the paper.

One additional finding of unknown significance should be reported here. As is obvious from figure 1 epicotyls inverted into test solutions and subsequently reinverted into new media suffered a necrosis and shrinkage of the previously immersed apical portion. However, if 50 γ/ml. NAm were present in the test solution bathing the immersed area, no shrinkage resulted, and in some instances, an actual enlargement was produced. This

effect of NAm, unlike the bud growth effect, is independent of IAA and of illumination.

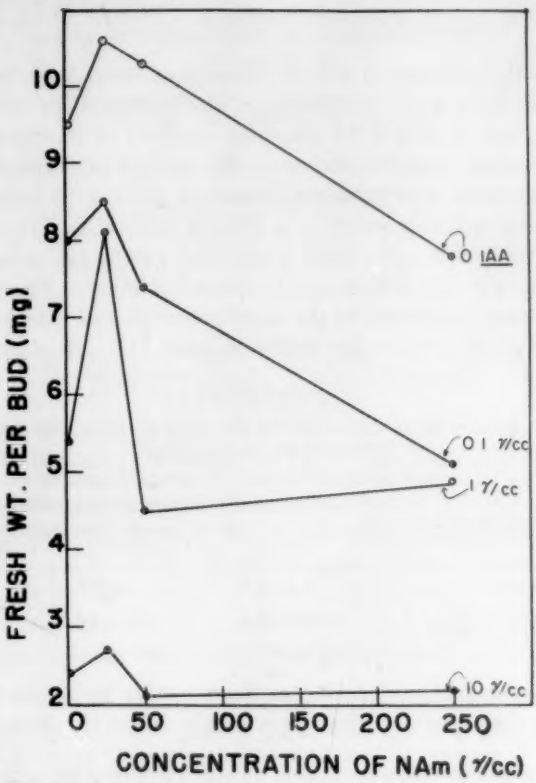

FIG. 2. Quantitative interaction between NAm and IAA in the bud growth test.

MECHANISM OF THE INTERACTION

When one attempts to explain this type of interaction between IAA and NA, one may make a working hypothesis on the basis of recent biochemical findings. For instance both IAA (**16**) and NA (**9, 10**) are known to arise from tryptophan in various organisms. The transformations leading to IAA production from tryptophan apparently proceed *via* tryptamine or indolepyruvic acid (**8**), whereas those leading to NA production from tryptophan proceed *via* kynurenine and 3-hydroxyanthranilic acid (**2, 11**). Since both IAA and NA may arise from tryptophan, and since both are physiologically active, the synergistic effect could be explained in terms of a sparing action. Thus, the addition of exogenous NA would make possible a greater diversion of tryptophan to IAA, resulting in an apparently enhanced IAA effect. To test the applicability of this hypothesis to the

etiolated pea plant, it was necessary to conduct experiments to determine whether the hypothecated conversions actually do occur.

The conversion of tryptophan to auxin

Epicotyls were harvested and cut into pieces about 1 cm. long. Aliquots of the pooled tissue were then removed and incubated in control solutions (2% sucrose and M/15 pH 6.1 phosphate buffer) or in experimental solutions (the same solution fortified with 250 mg/L 1(−)tryptophan). After 16 hours, the tissue was gathered, rinsed 3 times with redistilled water, frozen in liquid air, and dried in a lyophil apparatus. The dried tissues were ground to 40 mesh in a small Wiley mill, and 50 mg. samples extracted for two hours with ice-cold peroxide-free ethyl ether. The ether extracts were then assayed for auxin by the conventional Avena coleoptile curvature test (**18**). Typical results are shown in table III. It is quite clear that

TABLE III

The conversion of 1(−)tryptophan to auxin by etiolated pea tissue. Description in the text

Treatment	Degrees curvature ± standard error		
	Row A	Row B	Average
Control	2.2 ± 0.5	2.1 ± 0.3	2.2
+ 250 mg/L 1(−)tryptophan	11.9 ± 1.0	19.4 ± 1.0	15.7

the addition of tryptophan to the medium results in higher ether-extractable auxin in the tissues. This is presumably due to the formation of IAA.

The conversion of precursors to nicotinic acid

Epicotyl tissue was cut into 1 cm. lengths and 500 mg. fresh wt. immersed in 10 ml. of solution containing a presumed precursor. The following concentrations were used: 1(−)tryptophan 250 mg/L; 1-kynurenine sulphate 100 mg/L; 3-hydroxyanthranilic acid 50 mg/L. After 24 hours, the tissue was removed, rinsed, covered with 1 N H_2SO_4 and hydrolyzed in an autoclave for 30 min. at 15# pressure. After cooling, the hydrolyzate was filtered free of tissue fragments, adjusted to pH 6.8 and made up to standard volume. Aliquots were then assayed for nicotinic acid.

In the assay, 5 ml. of NA-free medium was added to 5 ml. of solution to be assayed, then inoculated with one drop of a 24-hour old culture of *L. arabinosus* 17–5. The tubes were incubated at 30° C for 72 hours, and the lactic acid titrated with 0.1 N NaOH, using bromthymol blue as an indicator. A standard series was run along with the unknowns, and nicotinic acid in each tube determined by reference to the standard curve. In general, the utilizable assay range was .05 – 0.4 γ nicotinamide per tube. Despite repeated trials, evidence could be found only for a slight conver-

sion of tryptophan to NA. However, kynurenine and especially hydroxyanthranilic acid seemed definitely to be readily converted to a material active as NA in the bioassay. Results are summarized in table IV.

TABLE IV

THE EFFECT OF VARIOUS PRESUMED PRECURSORS ON THE NA CONTENT OF PEA EPICOTYL TISSUE. DESCRIPTION IN THE TEXT

EXPERIMENT #	γ NA PER GM. DRY WEIGHT OF TISSUE				
	CONTROL	250 MG/L 1(−)TRYPTOPHAN	100 MG/L 1-KYNURENINE SULPHATE	50 MG/L 3-HYDROXY-ANTHRANILIC ACID	50 MG/L NA
P-256	45	55	71	225	259
P-243	84	89	100	194	207
P-158	33	33	.....	.....	400
P-152	33	50	.....	.....	150

PHYSIOLOGICAL EFFECTS OF IAA- AND NA-PRECURSORS

If tryptophan may be converted to IAA and to NA as indicated by the previous experiments, then it should also exert physiological effects produced by these end products. Similarly, kynurenine and hydroxyanthranilic acid should produce the same general effects as does NA. That these effects are in fact produced is evident from the following experiments.

Precursors were incorporated into test solutions in the concentrations

TABLE V

THE EFFECT OF PRESUMED PRECURSORS OF NA ON THE GROWTH OF ETIOLATED PEA EPICOTYL BUDS IN THE LIGHT. EPICOTYLS EITHER ERECT OR INVERTED DURING THE FIRST 24 HOURS; ALL ERECT DURING FINAL SIX DAYS; HARVEST AT END OF SEVENTH DAY

TEST SOLUTION	EPICOTYLS ERECT OR INVERTED DURING 1ST 24 HOURS	FRESH WEIGHT OF BUDS PER 10 EPICOTYLS (MG.)
Water	Inverted	67.3
500 γ/ml. 1(−)tryptophan	"	9.4
200 γ/ml. 1-kynurenine sulphate	"	92.0
100 γ/ml. 3-hydroxy-anthranilic acid	"	108.0
50 γ/ml. NAm	"	123.2
Water	Erect	19
500 γ/ml. 1 (−)tryptophan	"	81
50 γ/ml. NAm	"	84

indicated. The usual bud growth test was performed, all flasks being exposed continuously to 500 fc of light. After seven days, the buds were excised and weighed. Data are presented in table V.

It is obvious that kynurenine, hydroxyanthranilic acid and nicotinamide, when applied apically, all stimulated bud growth. Tryptophan when applied apically was inhibitory, but when applied basally was stimulatory; indicating that it may produce both IAA and NA. Under conditions where IAA may migrate to the bud (basipetally) the net effect is an inhibition: under conditions where IAA cannot migrate to the bud (acropetally) the net effect is stimulation. In subsequent experiments, it was also shown that tryptophan may result in enhanced root initiation, further indicating that the tryptophan ⟶ IAA transformation does occur.

Discussion

On the basis of the data presented it seems valid to conclude that (a) various physiological interactions between IAA and NA may be demonstrated in the etiolated pea plant and (b) these interactions are probably due to a sparing action, since both NA and IAA are produced from a common precursor, tryptophan (figure 3). Thus, the addition of either one

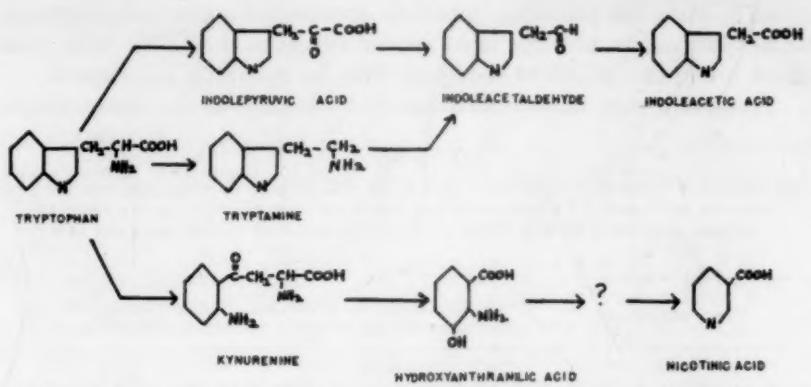

FIG. 3. Summary of pathways of conversion of tryptophan to nicotinic acid and to indoleacetic acid.

makes available more tryptophan for conversion to the other, effectively increasing the concentration of both constituents by the addition of one. Whether synergism or antagonism is to result from such an interaction will depend on the ratio of these two products formed and upon the organ involved.

AUDUS (1) reports an inability to detect IAA-NA interactions in seedlings of water cress. Since the tested seedlings were evidently connected with their cotyledons, it seems that failure to obtain positive results stemmed from a failure to deplete endogenous reserves of both NA and IAA.

Under such conditions, addition of small quantities of exogenous NA would not be expected to yield discernible results.

It should also be noted that although TERROINE (**14**) reports an inability of cultured *Phaseolus multiflorus* embryos to form NA from tryptophan, NASON (**12**) reports success in a similar experiment with *Zea mays*. In neither case did the experimenter attempt to utilize any intermediates between tryptophan and NA.

It may be significant for the problem of lateral bud inhibition that a single substrate (tryptophan) may be metabolized to two different end products, one of which (NA) may stimulate bud growth and the other of which (IAA) inhibits bud growth. It would appear advisable to conduct similar investigations on other plants, to see whether the inhibition imposed by IAA may be reversed by proper levels of NA or other bud growth factors. In this connection, it is necessary to recall that previous investigations (**6**) have demonstrated that adenine may also serve as a bud growth factor in etiolated peas. NA may apparently substitute for adenine; the maximum rate of bud growth produced by either of these materials is not further increased by the addition of the other. The mechanism of this effect is as yet not clear; but it is apparent that the demonstration that presumed NA precursors may stimulate bud growth is not definitive proof of their conversion to NA. However, when such evidence is supported by evidence from microbioassays, there can be little doubt that the transformations actually do occur.

Summary

Various physiological interactions between indoleacetic acid (IAA) and nicotinic acid (NA) have been demonstrated in the etiolated pea epicotyl. For example, NA enhances the root initiation activity of IAA (synergism), but IAA reverses the bud-growth stimulation produced by NA (antagonism). Evidence from bioassays indicates that tryptophan may be converted rapidly to IAA and slowly to NA, the latter conversion apparently occurring through kynurenine and 3-hydroxyanthranilic acid as intermediates. It is suggested that the various IAA-NA interactions may be explained on the basis of their origin from a common precursor, tryptophan.

The author wishes to express appreciation to Miss Margery Hand for aid rendered during the course of this investigation.

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THE EFFECT OF ONION JUICE ON THE GROWTH RESPONSE TO AUXIN

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(WITH NINE FIGURES)

Received June 8, 1949

Introduction

After the report of LUCAS and HAMNER (**9**), that the juice of onions had an effect in promoting the activity of 2,4-D, it became of interest to identify the factor responsible for this increased activity. Lucas and Hamner used 1,000 p.p.m. of the sodium salt of 2,4-D dissolved in aqueous extracts of Michigan Sweet Spanish Onions and measured the effect by the increase in weight of a Red Kidney Bean leaf over that of the leaf opposite to it, when 0.05 ml. of the mixture was applied to the petiole. They found an optimum effect when the onion juice was diluted to 1:20 or 1:30 and was either neutral or acid. If diluted to 1:50 or if it was made alkaline, the activating effect of the onion juice was lost. Lucas and Hamner stated that the effect was to make a given dose of the 2,4-D salt as effective as higher doses of the salt, or equal to that of the same quantity of the acid or its esters.

From time to time treatments have been reported which apparently increase the effectiveness of a given dose of auxin. Thus mixtures of two different auxins have been shown to have effects greater than either one alone (**1, 5, 8, 17**). Substances chemically related to auxin but themselves inactive were shown to increase the curvatures produced by auxin on slit pea stems (**16**), and triiodobenzoic acid has recently been shown to act similarly in several tests (**12**). In addition, many other unrelated substances have been reported which somewhat increase the killing effect of 2,4-D (**6**). Although these phenomena may be basically heterogeneous in nature, they can have great practical and theoretical importance.

The experiments described below were therefore undertaken to determine whether or not the effect of the onion juice is a fundamental effect on growth as a response to auxin, and to determine the nature of the active principle.

Materials and Methods

The onion juice was prepared by peeling Ebenezer sweet yellow onions* and grinding them for about 15 to 20 minutes in a Waring blendor. The resulting mash was then filtered, and the filtrate centrifuged until the supernatant liquid was clear; this fluid was decanted off and acidified to about pH 2. The juice was next extracted three times with freshly dis-

* The juice of white onions was about equally effective.

tilled ether in order to remove any auxin present. The residual ether was then driven off by heating for several hours in a hot water bath. The pH was adjusted to 6.0 and the extracted onion juice was stored in the refrigerator until used. As an example, 277 g. peeled onions yielded 198 g. juice.

The ether extract was evaporated just to dryness, and the residue taken up in 5 ml. of distilled water and stored in a refrigerator. The activity of this fraction was subsequently tested as described below.

Most of the experiments were carried out with the curvature response of the slit third internodes of seven-day-old pea stems (**15**). The curvature was determined as described by THIMANN and SCHNEIDER (**14**) by measuring the amount of negative or positive curvature (in degrees) that the tangent to the tip of each of the arms makes with the axis of the basal unslit portion. A series of experiments was also performed to determine the effect on straight growth of 20 mm. sections of seven-day-old pea stems. *Pisum sativum* var. Alaska was used throughout the experiments. For both the curvature and straight growth experiments, the stem sections were placed in 9 cm. Petri dishes containing 20 ml. of solution. Each pea curvature treatment consisted of five to seven stem sections (10–14 halves), and each of the straight growth treatments consisted of 15–20 20 mm. sections. The plants were usually placed in the test solutions after being soaked for two to five hours in tap water to remove any residual auxin (see below), and the results were measured about 36 hours later. Both indole-3-acetic acid and 2,4-dichlorophenoxyacetic acid were used as auxins.

The reducing sugars were determined by the method of SHAFFER and SOMOGYI (**11**). Total sugars were determined as reducing sugars after hydrolysis with 5 ml. concentrated HCl in a 50 cc. aliquot of the juice. Phosphates were determined by the method of FISKE and SUBBAROW (**4**), the total phosphates being obtained by first hydrolyzing the juice with 1N H_2SO_4 for 180 minutes at 100° C.

Preliminary Experiments

DISTRIBUTION OF THE ACTIVITY

The ether-insoluble fraction was found to be very effective in promoting the activity of both 2,4-D and indole-acetic acid. The results with this fraction will be described in more detail below. The ether-soluble fraction had some slight promotion effect, but this could easily have been due to the solubility of the water fraction in the ether. Since the activity of this fraction was not over 5 per cent. of that of the ether-insoluble part, it was not further studied.

LOSS OF ACTIVITY OF ONION JUICE

It was found that the ether-extracted juice decreases in activity on standing, even though kept in a refrigerator. Generally it was not used

after six weeks. There was only a slight decrease in activity after autoclaving but because of the short duration of the experiments it was not considered necessary to autoclave the test solutions.

The effect of pre-soaking pea sections in water

Went (**16**) drew attention to the small but definite curvature often produced by substances chemically related to auxins, when the slit pea stems were placed immediately into test solutions. These curvatures were ascribed to activation of the residual auxin in the stem, and were prevented by pre-soaking the slit sections in water for two to four hours.

It was found in the present experiments that the ether-extracted onion juice had some effect in decreasing the negative curvature of the slit pea stem when no auxin was added. If, however, the sections were soaked in water for two hours before being placed in the test solutions the effect disappeared. The ether-extracted onion juice therefore does not itself contain appreciable amounts of auxin, and the above effect can doubtless be interpreted as due to an action exerted on the residual auxin in the pea sections. In subsequent tests the sections were always soaked in water for two to four hours after slitting but before being placed in the test solutions.

Effect of concentration of onion juice

A concentration of 5 per cent. onion juice was found to be most effective, but this was frequently toxic to the pea sections. This agrees with the conclusion of Lucas and Hamner (**9**) that the optimum dilution was 1:20 to 1:30. Substantial increases in the effectiveness of indole-acetic acid and 2,4-D were, however, obtained with lower concentrations, down to 0.5 per cent. The finding of Lucas and Hamner that a dilution of 1:50 or 2 per cent. is ineffective may be due to differences in the sensitivity of the methods employed. Concentrations as dilute as 0.05 per cent. (1:2000) were found to have some effect on the pea sections in presence of high concentrations of 2,4-D and indole-acetic acid.

In all of the succeeding experiments it was decided to use a concentration of the ether-insoluble fraction corresponding to 2.5 per cent. of the original onion juice as the standard onion juice treatment.

The effect of onion juice on the response to auxin

Pea curvature test

Two and one-half per cent. onion juice was very effective in increasing the growth response to both indole-acetic acid and 2,4-D as measured by the curvature of slit pea stems. With indole-acetic acid (figure 1) the onion juice became more effective as the concentration of the auxin was increased. The data of figure 1 represent the mean of 13 complete ex-

periments, in all of which the juice definitely increased the curvature. When 30 mg./l. of indole-acetic acid was used, 2.5 per cent. added onion juice gave an increased curvature averaging 130° above that of the controls.

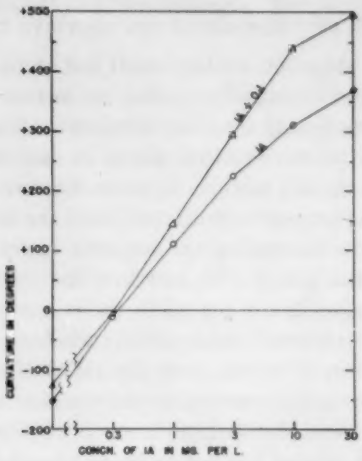

FIG. 1. Curvature of slit third internodes of seven-day-old etiolated Pisum stems ("pea test") in indole-acetic acid alone (IA), and indole-acetic acid plus 2½ per cent. extracted onion juice (IA + O.J.). The average of 13 complete experiments. In this and all subsequent figures the scale of auxin concentration is logarithmic.

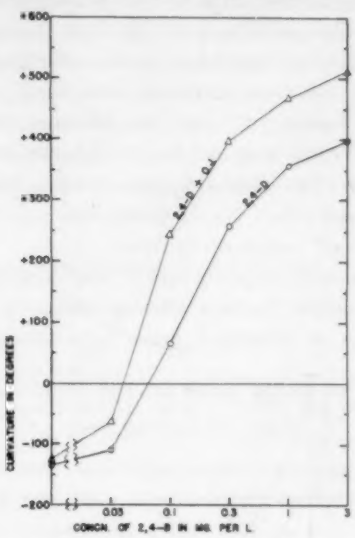

FIG. 2. "Pea test" in 2,4-D alone (2,4-D), and 2,4-D plus 2½ per cent. extracted onion juice (2,4-D + O.J.). The average of eight complete experiments.

When 2,4-D was used as the auxin, the onion juice had a more nearly constant effect in increasing the activity of the growth substance (figure 2)

particularly in the range 0.1 to 3.0 mg./l., with the increased curvature ranging from 110° to 180° over that of the controls. Figure 2 represents the mean of eight complete experiments, in every one of which the juice definitely increased the curvature response to 2,4-D.

Straight growth of pea sections

The data presented in table I indicate that 2.5 per cent. onion juice

TABLE I

THE EFFECT OF ONION JUICE ON STRAIGHT GROWTH.
PERCENTAGE ELONGATION (IN 36 HOURS) OF 20 MM. SECTIONS OF THE THIRD INTERNODE OF ETIOLATED SEVEN-DAY-OLD PISUM STEMS.

CONCENTRATION OF AUXIN (mg./l.)	0	0.1	0.3	1.0	3.0	10.0	30.0
	Indole-acetic acid (each value the mean of 52 sections)						
Elongation when solutions were made up with:—							
Distilled H_2O	14.6	.....	33.4	37.0	42.2	46.9	49.8
2½% Extracted onion juice	18.0	.....	34.5	43.4	45.8	51.4	56.0
	2,4-D (each value the mean of 13 sections)						
Elongation when solutions were made up with:—							
Distilled H_2O	.....	24.8	35.4	43.4	48.1	.....	.....
2½% Extracted onion juice	.....	27.5	39.4	49.8	57.9	.....	.....

has some small effect in increasing the growth-promoting activity of indole-acetic acid and 2,4-D when measured by the change in length of 20 mm. pea sections. The data for indole-acetic acid are the average of three experiments, in each of which the onion juice had a small but significant effect in increasing the elongation. The data for 2,4-D are from a single experiment with thirteen 20 mm. sections per dish. The somewhat lower effectiveness of onion juice when measured by this technique is probably due to a difference between the tissues being measured by the two methods. In straight growth measurements the entire stem response is being determined, including the less responsive inner tissues which decrease the sensitivity of the test object. In the curvature test the response of the sensitive outer tissues of the pea stem is the main factor being determined, while the retarding effect of the less responsive inner tissues is reduced to a minimum by the slitting. This phenomenon has been the subject of numerous experiments and discussions (**14, 3, 7, 16**).

Nature of the active principle

THIMANN and SCHNEIDER (**14**) noted in passing that 1 per cent. sugar, alone or with phosphate concentrations of 1×10^{-5} M to 0.1 M, was not generally effective in increasing the growth of Pisum sections in auxin, although sucrose alone was, of course, effective in producing an increase in

the growth of sections of Avena coleoptiles. In order to exclude the possibility that the effect of onion juice was merely due to the sugar, or sugar and phosphate, present in the juice, a series of experiments was undertaken.

The onion juice was first analyzed to determine the amount of reducing and total sugars and phosphate present; the results in terms of 2.5 per cent. onion juice are presented in table II. These results are in agreement

TABLE II

SUGAR AND PHOSPHORUS CONTENT OF THE JUICE OF EBENEZER SWEET YELLOW ONIONS. DATA REFER TO SAMPLES VOLUME 5 CC. EACH

CONSTITUENT	SAMPLE	EXPERIMENTAL DATUM	CALCULATED CONCENTRATION IN 2½% ONION JUICE
REDUCING SUGAR	Before ether extraction; diluted 1:400	0.62 mg. "glucose"	0.124%
	After ether extraction; diluted 1:400	0.70 mg. "glucose"	0.14 %
TOTAL SUGAR	Before ether extraction; diluted 1:400	0.725 mg. "glucose"	0.145%
	After ether extraction; diluted 1:400	0.727 mg. "glucose"	0.145%
SOLUBLE P	Sample I; diluted 1:40	0.015 mg. P	0.97×10^{-4}M
	Sample II; diluted 1:40	0.0407 mg. P	2.6×10^{-4}M
TOTAL P	Sample II; diluted 1:40	0.0477 mg. P	3.1×10^{-4}M

with those obtained for yellow onions by DAHLEN (**2**) and others (cited in **18**) whose data correspond to 0.15 per cent. reducing sugar and $1.23–2.29 \times 10^{-4}$ M phosphate for 2.5 per cent. yellow onion juice.

These concentrations of sugar and phosphate were therefore added to auxin solutions in the form of dextrose and pH 6 potassium phosphate

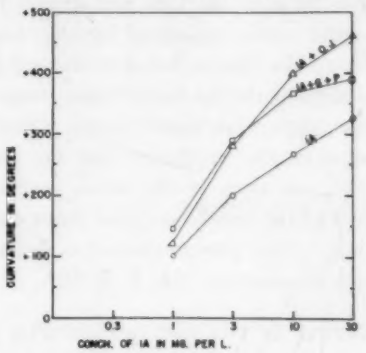

FIG. 3. "Pea test" in indole-acetic acid alone (IA), indole-acetic acid plus 2½ per cent. onion juice (IA + O.J.), and indole-acetic acid plus 0.19 per cent. glucose with 1.76×10^{-4}M or 3.52×10^{-4}M phosphate (IA + G + P). The average of five complete experiments.

buffer. The resulting curvatures were compared with those produced by 2.5 per cent. onion juice plus auxin, and by auxin alone. The results are presented in figures 3 and 4. Each of these figures represents the mean of

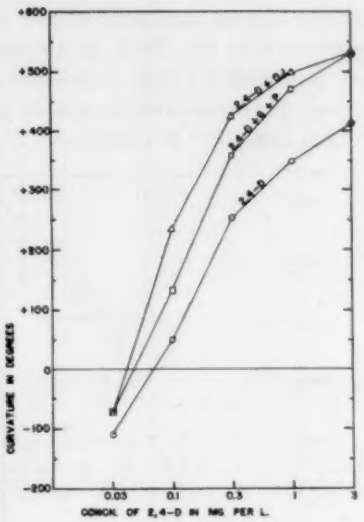

FIG. 4. "Pea test" in 2,4-D alone (2,4-D), 2,4-D plus 2½ per cent. onion juice (2,4-D + O.J.), and 2,4-D plus 0.19 per cent. glucose with 1.76×10^{-4}M to $3.52 + 10^{-4}$M phosphate (2,4-D + G + P). The average of five complete experiments.

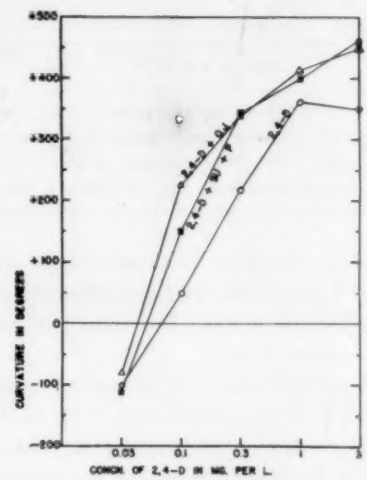

FIG. 5. "Pea test" in 2,4-D alone (2,4-D), 2,4-D plus 2½ per cent. onion juice (2,4-D + O.J.), and 2,4-D plus 0.19 per cent. sucrose with 1.76×10^{-4}M phosphate (2,4-D + Su + P). The average of two complete experiments.

five complete experiments, with essentially similar results. It can readily be seen that the concentrations of glucose and phosphate that are present in the 2.5 per cent. onion juice are indeed effective in increasing the activity, both for indole-acetic acid and for 2,4-D. While in general the effect approaches the magnitude of that produced by the onion juice, it will be seen that with indole-acetic acid the effect falls short of that of the juice at high concentrations and with 2,4-D it falls short in the middle range.

Similar concentrations of sucrose and phosphate (0.15 to 0.30 per cent. sucrose and 9.7×10^{-5} M to 1.76×10^{-4} M phosphate) are also effective (figure

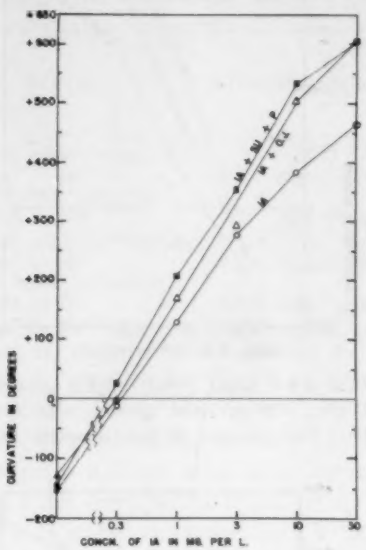

FIG. 6. "Pea test" in indole-acetic acid alone (IA), indole-acetic acid plus 2½ per cent. onion juice (IA + O.J.), and indole-acetic acid plus 0.3 per cent. sucrose with 4×10^{-3}M phosphate (IA + Su + P). The average of three complete experiments. The "IA + O.J." curve may be compared with that of figure 1, which shows no deviation at 3 mg. per l., and comprises a much larger number of experiments.

5). The effect of the onion juice is satisfactorily duplicated. The combination of sucrose and phosphate was also effective in increasing the curvature response to indole-acetic acid, but the effect was somewhat short of that produced by the onion juice.

When a series of concentrations of phosphate and sucrose were tested it was found that 0.004 M phosphate with 0.10 to 0.30 per cent. sucrose was even more effective with indole-acetic acid than onion juice, 0.30 per cent. sucrose and 0.004 M phosphate giving the optimum effect. Figure 6 shows that with these concentrations the effect of onion juice can in general be exceeded.

The results of two experiments comparing the relative effectiveness of

sucrose and glucose, both at 0.3 per cent. with added 0.004 M phosphate, showed that the two sugars were about equally effective (figure 7). Without added phosphate, 0.3 per cent. sucrose had only a small effect in increasing the curvature, while 0.004 M phosphate without added sugar had a

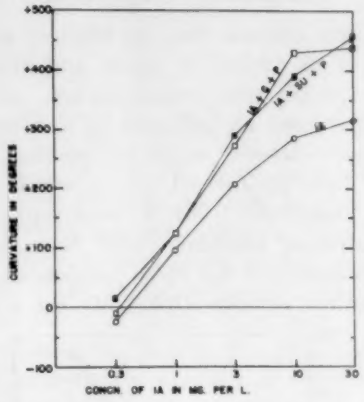

FIG. 7. "Pea test" in indole-acetic acid alone (IA), indole-acetic plus 0.3 per cent. glucose with 4×10^{-3}M phosphate (IA + G + P), and indole-acetic acid plus 0.3 per cent. sucrose with 4×10^{-3}M phosphate (IA + Su + P). The average of two complete experiments.

somewhat greater effect. However, the optimum increase seems to be brought about only by the combined presence of both sucrose and potassium phosphate.

The combination of glucose and phosphate also definitely promotes straight growth of the pea sections. Thus, in 1 mg./l. indole-acetic acid

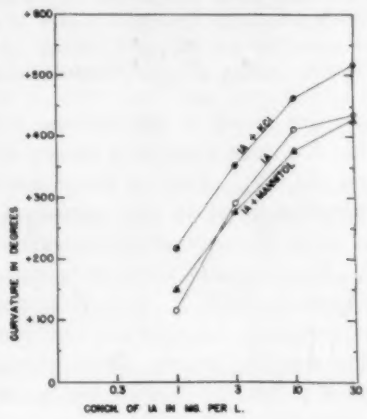

FIG. 8. "Pea test" in indole-acetic acid alone (IA), indole-acetic acid plus 0.011 M KCl (IA + KCl), and indole-acetic acid plus 0.021 M Mannitol (IA + Mannitol). The results of a single experiment.

the growth of the sections was increased from 32.2 per cent. in the auxin alone to 37.2 per cent. in the auxin with 2.6×10^{-4} M phosphate and 0.19 per cent. glucose. More extensive data will be given in a later paper.

The role of potassium ions

While the above data indicate that the effect of onion juice can be explained on the basis of its content of sugars and phosphate, they give no indication as to how specific these substances are. Although the effectiveness of sugars would suggest an influence on metabolism, it was thought possible that the effects observed might be essentially osmotic in nature. An experiment was therefore carried out in which the glucose (0.3 per cent. = 0.017 M) and phosphate (0.004 M) were replaced by a roughly corresponding concentration of mannitol (0.021 M). As shown in figure 8, mannitol cannot be substituted for these compounds. However, figure 8 also shows that potassium chloride (0.011 M) has a marked effect in in-

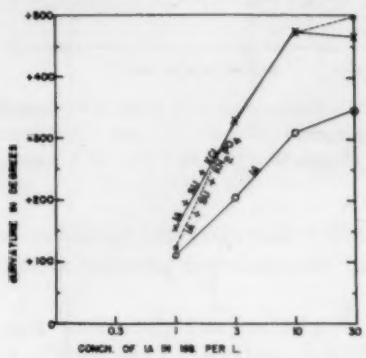

FIG. 9. "Pea test" in indole-acetic acid alone (IA), indole-acetic acid plus 0.3 per cent. sucrose with 4×10^{-3}M potassium phosphate buffer (IA + Su + KH_2PO_4 (dotted line)), and indole-acetic acid plus 0.3 per cent. sucrose with 4.2×10^{-3}M potassium chloride (IA + Su + KCl). The average of three complete experiments.

creasing the curvature due to auxin, the increase being from 50 to 100°. Potassium chloride has for some time been known to increase the elongation of Avena sections (**13**, **14**). Further experiments with the potassium ion at the same concentration as in the potassium phosphate-potassium hydroxide buffer, *i.e.* .0042 M, were therefore carried out in presence of sucrose, 0.3 per cent. The means of three such experiments, using indole-acetic acid, are presented in figure 9. It is clear that potassium chloride is as effective as the phosphate, *i.e.* that the increase in curvature is due to the potassium rather than the anion. Other experiments without sugar showed that the effects of potassium chloride and potassium phosphate alone were about the same, though in each case less than in presence of the sugar. Inasmuch as phosphate cannot be added without a cation, and since di- and tri-valent ions would introduce complicated and inhibiting effects on

growth (*cf.* **13**) further study of the role of phosphate was not pursued. It appears, however, that the principal effects here studied are those of sugars and potassium.

Discussion

The results show that as far as growth and curvature of the pea stem are concerned, the effect of onion juice can be ascribed almost wholly to its known constituents. The small deviation of the sugar and phosphate effect from that obtained with onion juice may possibly be due to difference in phosphate concentration of different onion juice samples and to differences in the inorganic ions present. Changes in phosphate concentration appeared to be much more critical than changes in the sugar concentration. The analysis of two different onion juice samples for phosphate showed that one had twice as much phosphate as the other. No attempt was made to analyze every batch of onion juice, and it is noteworthy that the onion juice sample with the higher phosphate concentration showed the better agreement between its effects and those of added sugar and phosphate of the same concentration (*cf.* the results presented in figures 3 and 4). Also the agreement was consistently best in those experiments in which analyzed onion juice samples were used, and therefore the exact quantity of potassium phosphate had been added. At the time the phosphate experiments were carried out the role of potassium was not appreciated, and it is possible that small quantities of other ions may be associated with the phosphate in onion juice. The present experiments by no means exhaust the effect of inorganic ions. Nevertheless it is clear that the effect of the onion juice can be very closely approached by using known concentrations of its simple constituents, *i.e.* sugar and potassium.

GALSTON and HAND (**4a**), in a paper which came to hand, after this manuscript was prepared, indicate that sucrose increases elongation of pea stem sections in auxin almost 50 per cent. However, since their experiments were all carried out in presence of phosphate buffer, the results are in agreement with ours. Galston and Hand did not compare the effects of other salts with that of phosphate.

The increased effectiveness of 2,4-D when used with buffered solutions as reported by LUCAS *et al.* (**9a**) may be explained in the light of these experiments, since every one of the buffers that they used contained potassium. In addition, their conclusion that the efficacy of the buffer system does not furnish an explanation for the increased efficacy of 2,4-D solutions prepared with onion extract is doubtless correct, but the postulate that another, as yet unknown, factor must be responsible for the action of onion juice seems now unnecessary.

MINARIK *et al.* (**10**) reported that the property of the onion juice which enhances the activity of 2,4-D when applied to the outer surfaces of plants appears to be due to some component which influences the penetration of

2,4-D into the leaf tissue. This was felt to be substantiated by experiments in which the 2,4-D, with and without onion juice, was injected directly into the cotyledons of bean seedlings. In this case, the presence of the onion juice in the injected solutions did not affect the plant's response to 2,4-D. Other solvents and certain wetting agents, such as Carbowax 1500, also enhance the activity of 2,4-D when applied to leaf surfaces, but not when injected. Minarik (personal communication) reports that onion juice has some surface activity.

The data presented above neither support nor refute the observations of Minarik *et al.*, but it would seem that if surface activity is a factor in the onion juice effect, it is of secondary importance. It is also possible that the effectiveness of surface active agents may be due to action of a quite different sort from that of the onion juice. The failure of the onion juice to exert any effect when inoculated could of course be due to the fact that the inoculations were made into the cotyledons, which are already well supplied with sugar, phosphate, and inorganic ions including potassium.

The results reported above indicate that a modification of the slit pea stem curvature test, adding 0.3 per cent. sucrose or glucose and 0.004 M potassium phosphate or potassium chloride to the test solutions, would markedly increase the sensitivity of the method. It also seems likely that it might be worth while to study the addition of sugar and phosphate, or of potassium chloride (*cf.* **8**), to the 2,4-D used as herbicide.

Summary

The reported effect of onion juice in enhancing the physiological activity of auxins on plants was studied using the curvature of slit pea stems and the straight growth of pea stem sections.

Onion juice does increase the growth-promoting activity both of indoleacetic acid and 2,4-D in these tests. The effect is not due to an auxin in the onion juice, and resides in the ether-insoluble fraction of the juice. Analysis of the juice demonstrated a considerable content of reducing sugar and phosphate, and when these constituents, in approximately the concentration in which they are present in the juice, are substituted for onion juice they have nearly the same effect. Sucrose was as effective as glucose and the optimum concentration of potassium phosphate was 4×10^{-3} M. Potassium chloride alone could duplicate more than half the effect of onion juice. The combination of potassium chloride and 0.3 per cent. sugar was about as effective as that of potassium phosphate and 0.3 per cent sugar, and either combination approximately duplicates the effect of the onion juice. Mannitol was ineffective as a substitute for sugars. It is concluded that the action of onion juice is due to its content of sugar, phosphate and potassium ions.

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RESPONSES OF PLANT CELLS TO HERBICIDES

H. B. Currier

(WITH TWO FIGURES)

Received May 14, 1949

In approaching the problems of weed killing through the techniques of cellular physiology, visible effects of four currently important herbicides on several types of plant cells were studied. Attention was focused on the cytoplasm for the most part. It is hoped that the over-all view of discernible cellular response obtained throughout a wide concentration range will provide a working basis for studies on more specific problems.

Methods

Tissues employed in this study and named below are classical materials of cellular physiology, chosen to represent different types of cells and to facilitate observation of cytoplasmic response.

outer (convex) bulb scale epidermis of *Allium cepa* (var. Red Creole)
leaves of *Anacharis canadensis*
storage parenchyma of *Beta vulgaris* root
root hairs of *Hordeum vulgare*
staminal hairs of *Tradescantia virginiana*

Following are the herbicides employed:

4,6-dinitro-2-sec. butyl phenol (DNSBP)
pentachlorophenol (PCP)
2,4-dichlorophenoxyacetic acid (2,4-D)
O-isopropyl-N-phenyl carbamate (IPC)

Aqueous solutions were prepared with ammonium salts of DNSBP and PCP and with the sodium salt of 2,4-D, ranging in concentration from 10^{-2} to 10^{-8} M. The 2,4-D salt additionally permitted preparation of 0.1 M. Aqueous solutions of IPC were not employed.

Paraffin oil solutions were prepared as follows: the parent compounds of DNSBP and PCP and the isopropyl ester of 2,4-D were used in the same concentration ranges as above; IPC was made up to 10^{-2} M, and a saturated solution ($<10^{-1}$ M) was also used.

Sections of Allium epidermis and Beta root, whole detached Anacharis leaves, 7-mm.-long root tips of Hordeum, and Tradescantia stamens were immersed in 5 ml. of aqueous solution in a squat vial for 24 hours at 20–23° C, then transferred to slides and cover slips ringed with thick paraffin oil. For all but root tips, other sections so treated were plasmolyzed by

mounting in 0.6 M KNO_3 solution, and after 30 minutes the type of plasmolytic response noted. For the paraffin oil treatment, the materials were mounted directly on slides and observed after 24 hours. While 24 hours was used as the standard duration time, subsequent observations were sometimes made.

Results

KILLING CONCENTRATION—There was considerable variation in the toxicity of the chemicals toward the different materials at the end of the specified 24-hour period (table I). The criterion of death was failure of the

TABLE I

MINIMUM CONCENTRATIONS OF THREE HERBICIDES IN AQUEOUS SOLUTION REQUIRED TO CAUSE DEATH OF THE VARIOUS TISSUES IN 24 HOURS AND UNDER THE CONDITIONS DESCRIBED

	MOLES PER LITER		
	NH_4-DNSBP	NH_4-PCP	Na-2,4-D
Allium epidermis	10^{-2}	10^{-3}	10^{-1}
Anacharis leaf	10^{-3}	10^{-3}	10^{-1}
Hordeum root hairs	10^{-3}	10^{-4}	10^{-2}
Tradescantia stamen hairs	10^{-3}	10^{-5}	10^{-3}
Beta root parenchyma	10^{-3}	10^{-4}	10^{-1}

cells to plasmolyze. Root and stamen hairs were more easily killed than the other cells. PCP was generally somewhat more toxic than DNSBP; 2,4-D was much less so, approximately 1/100 as effective.

STAGES IN THE KILLING PROCESS—An attempt is made here to arrange the various cellular responses into three phases: 1) "stimulation," 2) "retardation" (reversible injury), and 3) "premortal" (irreversible injury). This is shown by table II, while table III indicates in an approximate way the concentration ranges involved. There is overlapping between the phases, and it is possible that some effects may be misplaced. Similar terminology and phasic separation were employed by NORTHERN (**6**) in his study of auxin effects on the structural viscosity of protoplasm. Photomicrographs selected to represent typical responses are shown in figures 1 and 2.

STREAMING—An increase in the rate of protoplasmic streaming due to the stimulating action of various agents is well known (SEIFRIZ, **7**). The phenomenon is understood to be affected by a changing viscosity of the cytoplasm, which in turn may be associated with variable hydration. This effect was distinct in Anacharis and Tradescantia, and was produced in the stimulating concentration region. Comparison was difficult in the other materials. Toward the end of phase 2, the rate decreased, became jerky and erratic, and ceased in phase 3. There was some evidence of adaptation, *i.e.*, movement at first stopped but began again, moving slowly. Tra-

descantia stamen hairs when uninjured never failed to exhibit streaming, and cessation of movement was one of the first indications of injury.

PERMEABILITY—Decreased permeability over control experiments, in phase 1, was evidenced by a prolonged deplasmolysis in Allium, Anacharis,

TABLE II

LIST OF RESPONSES OBSERVED RESULTING FROM HERBICIDAL ACTION, AND GROUPING INTO THREE PHASES. COMPARISONS REFER TO CONTROL SECTIONS

RESPONSE	STIMULATION	REVERSIBLE INJURY	IRREVERSIBLE INJURY
Streaming rate	increased	decreasing, erratic	no streaming
Deplasmolysis time	decreased	increasing later	rapid, or not at all
Leakage of vacuolar pigment	decreased	increasing	marked
Protoplasmic granules	smaller	increasing in size	distinct and larger
Plasmolysis form	convex, more so than control	concave or cramped	irregular due to rigidity of ectoplast
Vacuolar contraction	not common	common	absent, or with cytoplasm vacuolated
Cap plasmolysis	more than in control	common	absent, or with cytoplasm vacuolated
Stimulation plasmolysis	occasional and slight	occasional and slight	persistent
Vacuolation	temporary	temporary	persistent, advanced
Shrinkage of whole protoplast	absent	absent	occasional
Survival time	greater than control	less than control	much less than control

TABLE III

APPROXIMATE CONCENTRATION RANGES OF THREE HERBICIDES, IN AQUEOUS SOLUTION, CORRESPONDING TO FOUR PHASES OF RESPONSE INDICATED

	MOLES PER LITER					
	ONION			ANACHARIS AND BEET		
	NH_4-DNSBP	NH_4-PCP	Na-2,4-D	NH_4-DNSBP	NH_4-PCP	Na-2,4-D
Stimulation	10^{-5} to 10^{-6}	10^{-6} to 10^{-7}	10^{-4} to 10^{-5}	10^{-6}	10^{-6}	10^{-4}
Retardation	10^{-4} to 10^{-5}	10^{-5}	10^{-3}	10^{-5}	10^{-5}	10^{-3}
Pemortal	10^{-3}	10^{-4}	10^{-2}	10^{-4}	10^{-4}	10^{-2}
Killing	10^{-2}	10^{-3}	10^{-1}	10^{-3}	10^{-3}	10^{-1}
	Stamen hairs			Root hairs		
Stimulation	10^{-7}	10^{-7}	10^{-5} to 10^{-6}	10^{-7}	10^{-6}	10^{-5} to 10^{-6}
Retardation	10^{-6}	10^{-6}	10^{-5}	10^{-6}	10^{-7}	10^{-4}
Pemortal	10^{-6}	10^{-6}	10^{-3} to 10^{-4}	10^{-6}	10^{-7}	10^{-3}
Killing	10^{-5}	10^{-5}	10^{-2}	10^{-5} to 10^{-6}	10^{-6} to 10^{-7}	10^{-3} to 10^{-2}

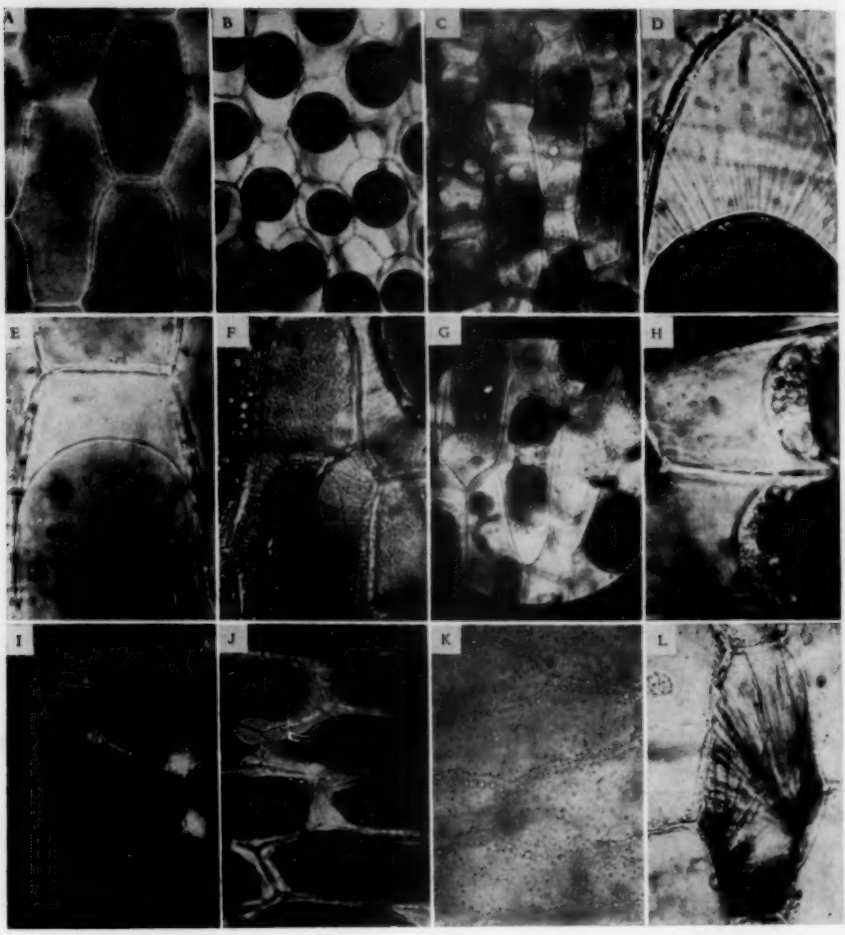

FIG. 1. Various responses of Allium epidermis. Unless specified, time of herbicidal treatment was 24 hours, and where plasmolyzed, tissues were treated with 0.6 M KNO_3 for 30 minutes. (A) Untreated. ×328. (B) Pronounced convex plasmolysis with KNO_3. ×172. (C) A degenerative type of "cramped" plasmolysis, 10^{-5}M Na-2,4-D, then KNO_3. ×182. (D) Adhesion, fine cytoplasmic strands connect cytoplasm and wall, 10^{-5}M Na-2,4-D, then KNO_3. ×885. (E) Vacuolar contraction, light area at top of cell is swollen cytoplasm, 10^{-7}M Na-2,4-D 96 hours. ×652. (F) "Tonoplast plasmolysis," cytoplasm disorganized but no actual separation from wall, 10^{-5}M Na-2,4-D, then KNO_3. ×513. (G) "Cap" plasmolysis, showing tendency of protoplast to divide, 10^{-5}M Na-2,4-D, then KNO_3. ×190. (H) Cap plasmolysis where "caps" have become markedly granular and vacuolated. ×577. (I) Stimulation plasmolysis approaching vacuolation, 10^{-5}M NH_4-DNSBP. ×370. (J) Marked adhesion after one hour in 10^{-5}M NH_4-DNSBP, then KNO_3. ×310. (K) Dispersion of cytoplasm into small droplets or granules by 10^{-2}M NH_4-DNSBP. ×293. (L) Crystals formed within cell after treatment with NH_4-PCP. ×382.

and Beta; also by decreased exosmosis of anthocyanin pigment from Beta. Cubes of root tissue showed less leakage in the lower herbicidal concentrations than in twice distilled water. In other tests, especially with root and stamen hairs, where the cells were treated with paraffin oil solutions there was less leakage of sap into the more dilute solutions than into pure oil. In phases 2 and 3 permeability increased, becoming almost complete in the advanced premortal condition. There was considerable leakage of pigment (fig. 2A, B) and failure, in advanced stages, to desplasmolyze.

GRANULES—The size of protoplasmic granules was smallest in the stimulation range; the cytoplasm exhibited an almost transparent, glistening appearance. With increasing injury, and especially in the premortal phase, the cytoplasm became distinctly granular. One interpretation is that granule growth is due to or paralleled by a dehydration and association of cytoplasmic particles.

PLASMOLYSIS FORM—The outline or form assumed by the protoplast on plasmolysis may be used as an indication of the degree of adhesion to the cell wall, as a measure of the hydration and viscosity of the cytoplasm, and the flexibility of the cytoplasmic membranes (WEBER, **8**; KÜSTER, **4**). Allium, Beta, and Anacharis are especially suitable here. There is little doubt that the convex form is characteristic of normal mature cells of the type represented by the three tissues mentioned (fig. 1B). In phase 2 the form becomes concave or cramped (krampf), with marked adhesion of the cytoplasm to the cell wall (fig. 1J), and in phase 3 the form is usually quite irregular (fig. 2F). Often the protoplast displays a rough outline due to the rigidity developed by the cytoplasm, and in instances rupture of the protoplast occurs (fig. 2G).

VACUOLAR CONTRACTION—This response can be identified by a shrinkage of the vacuole and swelling of the protoplasm (figs. 1E, 2C, K). It has been extensively studied by Küster, who in 1926 named the phenomenon. The cause may be an increase in the imbibition pressure of the cytoplasm, but other explanations have been proposed. ARENS and DE LAURO (**1**) discuss the matter at length. In any case it supplies direct evidence that the volume of cytoplasm within a cell can vary significantly. Although it may easily be mistaken for plasmolysis, in vacuolar contraction there is no separation of the cytoplasm from the wall, so that it is fundamentally different from plasmolysis. Occurring spontaneously in hypotonic solution or even in distilled water as a result of some kind of stimulation, vacuolar contraction has been identified in many kinds of tissues (KÜSTER, **4**). The phenomenon was characteristic in all five of our materials (somewhat vague in root hairs) in the concentration range described as reversible injury. At first the swollen portion of cytoplasm is hyaline, becoming granular and vacuolated with time or with concentration of chemical. That the swelling can occur at the expense of vacuolar water was demonstrated

by observing the reaction in sections bathed with paraffin oil. The swelling of nuclei, especially in Anacharis, (fig. 2H) was detectable in this phase, a reaction probably related to vacuolar contraction.

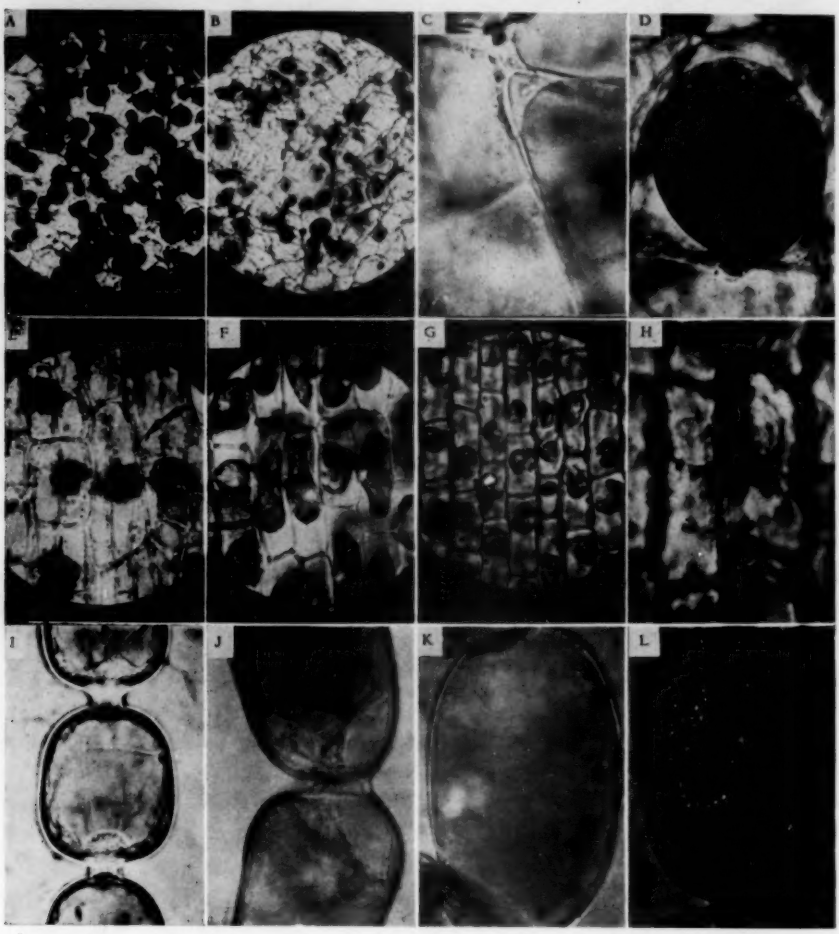

FIG. 2. Unless specified, time of herbicidal treatment is 24 hours; where plasmolyzed, 0.6 M KNO_3 was used for 30 minutes. A to D, Beta root cells; E to H, Anacharis leaf; I to L, Tradescantia hairs. (A) Normal convex plasmolytic response of untreated tissue to KNO_3 solution. ×87. (B) Necrotic aspect of similar section treated with 10^{-3}M NH_4-DNSBP, then KNO_3. ×87. (C) Incipient vacuolar contraction, 10^{-4}M DNSBP in paraffin oil. ×1190. (D) Cap plasmolysis, but similar to "systrophische Ballung" (KÜSTER, 4) 10^{-4}M Na-2,4-D, then KNO_3. ×667. (E) "Clumping" of chloroplasts, cells alive, saturated IPC in oil. ×350. (F) Irregular plasmolysis form, 10^{-3}M PCP in oil 48 hours, then KNO_3. ×350. (G) Apparent rupture of protoplasts, 10^{-3}M Na-2,4-D 48 hours, then KNO_3. ×178. (H) Swollen nuclei in slightly streaming cells, 10^{-4}M IP-2,4-D in oil 48 hours. ×700. (I) Swollen granular cytoplasm, streaming "erratically," 10^{-3}M PCP in oil. ×576. (J) Stimulative plasmolysis, 10^{-4}M DNSBP in oil 10 minutes. ×601. (K) Vacuolar contraction, cytoplasm fills upper part of cell, 10^{-3}M Na-2,4-D. ×710. (L) Coagulation pattern, 10^{-3}M NH_4-DNSBP. ×900.

STIMULATIVE PLASMOLYSIS—Here there is a spontaneous active contraction of the protoplast, resulting in the separation of cytoplasm from the cell wall (figs. 1I, 2J). The appearance is not different from ordinary plasmolysis, but, since it occurs in hypotonic solution, and so rapidly, the underlying mechanism cannot be a simple osmotic one. While not as characteristic of herbicidal action as vacuolar contraction in our observations, there was a greater amount of stimulative plasmolysis in the treated materials than in the controls. In many instances it is certain that reversible stimulative plasmolysis and vacuolar contraction had occurred in the first part of the treatment, but had disappeared after 24 hours.

VACUOLATION—The formation of vacuoles in the cytoplasm was of common occurrence. Sometimes it immediately followed immersion of the section in the herbicide solution, and usually the vacuoles would later disappear. Sudden transfer of tissues to KNO_3 solution also often produced this effect. Vacuoles appearing in the premortal phase were more persistent. Their size varied from one or two large vacuoles per cell, to dimensions so small that the cell assumed a frothy emulsoid appearance. The process of vacuolation may be interpreted as a dehydration of protoplasmic colloids. In agreement with LEPESCHKIN (5) it is more apt to occur where killing is a slow process, for some of the water that separates out is that taken up by an earlier swelling.

SURVIVAL TIME—Within the meaning of the term "stimulation" it is implied that the tissue has the ability to survive longer than control tissues. This would infer that the presence of herbicidal compounds in small amounts exerts some protective action, and tests running three and four days bear this out. However, it becomes less significant when the possible injurious effects of distilled water on the control cells are taken into account, despite the fact that such effects were not particularly noticeable.

MORE SPECIFIC REACTIONS—NH_4-DNSBP in 10^{-2} M (aq.) concentration produced a peculiar reaction in onion epidermis. In 10 to 15 minutes the cytoplasm in all cells began to disintegrate into small droplets, and after an hour the cytolysis was pronounced (fig. 1K), giving the section a white opaque appearance. Staining tests proved that the cytoplasmic layer actually had disintegrated, leaving only remnants, and that the effect was not the formation of an oily reaction product of the herbicide. The other tissues failed to show this response clearly, although there was some indication of it.

With NH_4-PCP in 10^{-3} M concentration, crystals formed within certain (not all) of the onion epidermal cells (fig. 1L). The cells so affected were dead or nearly dead by the time the crystals were observed. The unidentified crystals might be the relatively insoluble parent phenol precipitated by the acids of the sap.

Specific among the materials because of the presence of chloroplasts, Anacharis cells displayed aggregation or "clumping" of plastids in the retardation and premortal phases (fig. 2E).

Root hairs underwent plasmoptysis (bursting) to a greater extent when bathed by non-killing concentrations of herbicide than when placed in distilled water, but this behavior was quite variable. No bursting occurred in oil solutions, but there was slow leakage at the tip, and to a slight extent laterally, giving the hair a knotty appearance.

Results with oil solutions

In general, the paraffin oil solutions were one tenth as toxic as the aqueous solutions, and the consistency with which this relationship held was surprising. To interpret these results, one must consider the high oil-water partition coefficient, and the lower availability of oxygen under the experimental conditions, the latter presumed to cause a drop in metabolic activity. Relative toxicity therefore is not directly comparable with aqueous solutions.

Discussion

With some exceptions the cells studied reacted to the four herbicides in about the same manner at the same effective concentrations. It is noteworthy that the different compounds did not display individual reactions to any extent.

In the field, each of these substances may be used selectively, killing certain plants but not others. Selective action is believed to be conditioned both by morphological variation and by chemical and physical differences among species. In the present studies, where lethal concentrations differed among the various tissues, chemical and physical effects must be responsible. CRAFTS (**2**) has emphasized the point that selectivity is relative. Only when dosage, method of application, and weed and crop species are properly coordinated, do these chemicals kill weeds selectively.

While it is known that growth-regulating substances as 2,4-D may increase respiration and accelerate growth under certain conditions, such effects were not considered in this work, where relatively high concentrations were employed for short periods of time. Nor is reference made to the slow disorganization of meristematic tissue (VAN OVERBEEK, **9**) and cessation of growth characteristic of 2,4-D in dilute but lethal amounts. This probably relates to much more subtle reactions of the herbicide than those described in this paper, which must be considered for the most part to be the effects of strong contact poisons.

Most of the effects described are not peculiar to herbicides, but have been observed as a result of other means of stimulation and injury. This is in agreement with LEPESCHKIN'S (**5**) conclusions that morphological cellular changes during the course of killing may themselves be nearly unrelated to the kind of killing agent, whether physical or chemical in nature. Differences may exist in the rapidity of occurrence of various stages of injury, and to some extent in the relative sequence.

It is evident that most of the responses are hydration phenomena, in-

volving an increase or decrease in the water content of the cytoplasm. An increase is indicated for example by vacuolar contraction, cap plasmolysis, and nuclear swelling; a decrease by vacuolation, granulation, and coagulation. Rate of streaming, permeability changes, and plasmolysis form must be closely associated with degree of hydration. A discussion of possible mechanisms underlying these hydration phenomena may be found in CRAFTS *et al.* (**3**).

Summary

The visible effects of 2,4-dichlorophenoxyacetic acid, 4,6-dinitro-2-sec. butyl phenol, pentachlorophenol, and O-isopropyl N-phenyl carbamate on several types of tissues are described. The attempt is made to group the responses under the headings of (a) stimulation, (b) retardation (reversible injury), and (c) premortal (irreversible injury). Observation of vacuolar contraction, vacuolation, granulation, plasmolytic behavior and other phenomena indicate increasing cytoplasmic hydration during phase a, declining toward the end of phase b, and a marked decrease in c.

Acknowledgment is made to the Dow Chemical Company for supplying specially purified compounds used in this study, to Dr. R. W. Allard of the Davis Campus for a pure IPC preparation, and to Dr. J. C. Miller, Louisiana Agr. Expt. Station, Baton Rouge, for the supply of red onion bulbs.

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STUDIES IN THE METABOLISM OF CRASSULACEAN PLANTS: DIURNAL VARIATION OF ORGANIC ACIDS AND STARCH IN EXCISED LEAVES OF *BRYOPHYLLUM CALYCINUM*

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(WITH TWO FIGURES)

Received April 25, 1949

It has been shown in a previous paper (**7**) that the diurnal variation of the organic acid content of excised leaves of *Bryophyllum calycinum* cultured in water under normal light conditions in the greenhouse is accompanied by a diurnal variation of the starch content in the opposite sense. The acidity decreased during the day while the starch increased, but the acidity increased during the night while the starch decreased. In terms of the quantity of organic substances concerned, these two changes in composition were by far the largest observed, and their relative magnitude was such as to lend color to the view that the metabolism of organic acids in the leaves is closely connected with that of starch. The observations thus supported the hypothesis of BENNET-CLARK (**1, 2**) that certain of the polysaccharides and the malic acid in succulent plants of this type are the respective beginning and end products of a series of chemical equilibria, the relative concentration of these components at any point of time being a function of the conditions of illumination to which the leaves had previously been exposed. The observations of WOLF (**12, 13**) have laid particular emphasis upon starch as the polysaccharide concerned in the case of *Bryophyllum calycinum*.

The previous experiment carried out in this laboratory was limited to a study of the changes in composition of samples of *Bryophyllum calycinum* leaves cultured in water over a period of 24 hours. It seemed desirable, therefore, to repeat the experiment and study the changes for a longer period in order to see to what extent the chemical transformations would continue to respond to repeated alternations of light and darkness. Furthermore, a moderately accurate analytical method for isocitric acid has since become available, and direct examination of the behavior of this component is now possible. In the previous work, isocitric acid had been estimated by difference, a procedure that might well introduce error into the conclusions reached.

The present paper is, therefore, a report upon the composition of ex-

[1] Died Nov. 20, 1947.

[2] Died Nov. 20, 1948.

cised *Bryophyllum calycinum* leaves cultured in water in the greenhouse for three days with special attention to the diurnal changes in composition with respect to organic acids and starch.

Experimental

The samples were collected from plants derived from the same clone as those used in the earlier work and were grown under the same conditions. Small plants had been transplanted to sand in crocks on February 2, 1947. The collection was made at 4 A.M. (standard time) on July 7 (sunrise, 4.26 A.M.; sunset, 7.28 P.M.), the leaflets being cut from the upper 8 to 10 petioles from 28 plants. The individual samples were selected by the leaf size method (**10**), 11 samples of mean weight 262.2 ± 2.2 grams and including from 49 to 53 leaflets each being taken. Two of

TABLE I

FUNDAMENTAL DATA ON SAMPLES OF EXCISED *Bryophyllum calycinum* LEAFLETS SUBJECTED TO CULTURE IN WATER IN ALTERNATE LIGHT AND DARKNESS

	PER SAMPLE			PER KILOGRAM INITIAL FRESH WEIGHT				
CULTURE PERIOD	FRESH WEIGHT AT START	FRESH WEIGHT AT END	EQUILIBRATED CRUDE DRY WEIGHT	CRUDE DRY WEIGHT	NITROGEN	PROTEIN NITROGEN	ORGANIC SOLIDS	ISOCITRIC ACID
hrs.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*m.eq.*
0	266.7	266.7	29.8	111.7	2.00	1.62	88.6	168
0	260.5	260.5	28.5	109.4	2.00	1.73	88.7	170
10	261.0	253.6	29.3	112.3	2.04	1.67	90.4	173
16	260.0	250.4	28.2	108.5	1.97	1.66	87.6	178
24	260.6	258.3	28.3	108.6	2.13	1.74	87.4	173
34	261.8	256.9	28.6	109.2	2.07	1.67	87.7	174
40	261.1	256.2	30.1	115.3	2.02	1.64	93.0	172
48	265.3	263.8	29.7	111.9	2.04	1.61	90.2	171
58	261.1	259.0	28.3	108.4	2.08	1.69	86.9	160
64	262.1	260.0	29.6	112.9	2.05	1.63	93.1	166
72	264.1	263.2	31.5	119.3	2.10	1.72	93.3	171
Mean	262.2				2.045	1.67	89.7	170.5
Standard deviation	±2.2				±0.047	±0.045	±2.5	±4.7
Coefficient of variation (%)	0.83				2.3	2.7	2.7	2.8

these samples were at once dried for use as controls and the remainder were arranged in troughs in the greenhouse, the bases being immersed in distilled water. Single samples were removed and dried for analysis at 2 P.M., 8 P.M., and 4 A.M., (standard time) during the following 72 hours. The first day was clear and bright, the second was cloudy until 8 A.M. and then intermittently bright and cloudy for the rest of the day. The third day was moderately fine although there was a brief thunderstorm at noon.

As will become evident in the discussion of the starch and organic acid composition of the samples, the variations in light conditions from day to day appear to have had an influence upon the extent and rate of the changes that occurred.

The first five columns of table I show the fundamental data from which the factor is derived to convert the composition of each sample, as obtained by chemical analysis in terms of percentage of the crude dry weight, to grams per kilogram of initial fresh weight. This correction of the data is essential in order that the comparisons of the separate samples shall be valid, for it was only at the time of collection that they were all in the same metabolic condition.

The samples were selected with special care to secure uniformity in initial fresh weight. The coefficient of variation of the fresh weight was only 0.83 per cent. That a moderately satisfactory degree of uniformity in composition was also achieved is evident from the results for the total nitrogen (table I, column 6); the coefficient of variation was 2.3 per cent. This value could be improved upon only by the application of the statistical method of collection of the leaflets (**10**), a method which was not devised until after the present culture experiment was completed.

Results

Protein nitrogen

In view of the unfortunate error that was made in computing the protein nitrogen (**9**) in three of the earlier papers of this series (**6, 7, 8**), special attention was devoted to this component in the present experiment. The data (table I, column 7) show that no significant change in the quantity of protein took place during culture in alternate light and darkness for three days. The coefficient of variation was 2.7 per cent., which is negligibly greater than that for total nitrogen. No significant change in total nitrogen could occur under the present conditions of culture.

Organic solids

The organic solids of the tissues did not change significantly throughout the period studied. The coefficient of variation (table I, column 8) was 2.7 per cent., identical with that for protein nitrogen. One might anticipate that photosynthesis during a period of daylight would lead to a detectable increase in organic solids, for such an increase is easily demonstrable in tobacco leaves cultured under similar conditions (**11**). The present data show that the net changes in organic solids in Bryophyllum leaves from photosynthesis and respiration were smaller than the variation among samples.

Organic acids

The changes in the acidity of the leaves are shown in the top curve of figure 1 in which the pH is plotted. Starting at the strongly acid reac-

tion of pH 3.95 at dawn, the acidity diminished to pH 5.5 by 2 P.M. and remained essentially unchanged in the sample collected shortly after sunset. During the night, the acidity increased to pH 4.1, but the cloudy weather of the second day appears to have interfered to some extent with

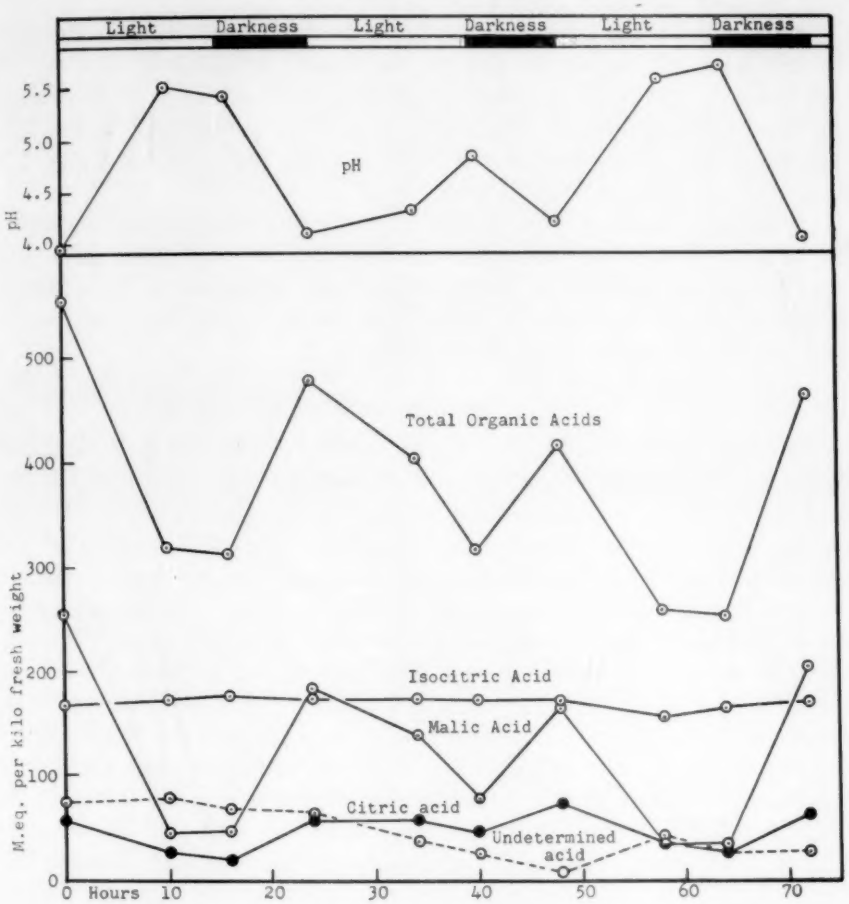

FIG. 1. Diurnal variation in pH (upper part of figure), total organic acids and individual organic acids of excised leaves of *Bryophyllum calycinum* cultured in water. Alternations of light and darkness indicated at top of figure. Data are milliequivalents of acid per kilogram of initial fresh weight.

the subsequent rise in pH; the maximum of 4.8 was not attained until the evening sample was taken. During the third day, however, the changes were quite similar to those of the first day.

The curve for total organic acids shows corresponding fluctuations; starting at 557 m.eq. per kilo of initial fresh weight at dawn, the acids had decreased by 244 m.eq. at sunset. The greater part of the loss arose from the disappearance of 213 m.eq. (14.3 grams) of malic acid, the remainder being citric acid which diminished by 37 m.eq. (2.37 grams).

During the night, the acids increased by 166 m.eq. most of which was malic acid although citric acid rose to its initial level. The decrease in acids during the second day was less precipitous than on the first, possibly because of the cloudy weather. Furthermore, citric acid diminished by only 14 m.eq. and malic acid by only 107 m.eq.

During the third period of daylight, the total organic acids dropped by 164 m.eq. and again the change was nearly complete by 2 P.M. Of this quantity, 135 m.eq. consisted of malic acid and 43 m.eq. of citric acid; together, these amount to 13.7 grams of organic substance. During the last night of the experiment, the acids rose by 212 m.eq. of which 173 m.eq. (11.6 grams) were malic acid.

It is clear, therefore, that excised Bryophyllum leaves retain the capacity to undergo diurnal variation in acidity for at least three days, and that the greater part of the change arises from alterations in the quantity of malic acid in the tissues. Citric acid shares in the variation but to only a moderate extent in relation to malic acid.

Isocitric acid

Especial significance attaches to the results for isocitric acid plotted in figure 1. The analytical method used depends upon the fact that isocitric acid is converted to citric acid by the enzyme aconitase present in mammalian heart muscle. Krebs and Eggleston (**5**) have shown that the equilibrium mixture of acids contained in a properly buffered system incubated with heart muscle aconitase contains 89.5 per cent. of citric acid, 6.2 per cent. of isocitric acid, and 4.3 per cent. of aconitic acid. Accordingly, as these authors have shown, isocitric acid can be determined from the difference in citric acid content of a sample of tissue before and after treatment with an extract or suspension of heart muscle. This method was applied by Krebs and Eggleston to several succulent species and gave results which indicated that isocitric acid shares to a small extent in diurnal changes in acidity, although malic acid was found to be the main acid component that altered in concentration. Bonner and Bonner (**3**) have employed a similar method in their studies of the effect of various partial pressures of carbon dioxide in the air upon the changes in acid content of several succulent species.

The curve for isocitric acid (figure 1) shows that this component of the tissues did not change significantly during the period of the experiment; the results[3] given in detail in table I (last column) yield a coefficient of variation of 2.8 per cent., only slightly greater than the variation in total nitrogen. Within the limits of accuracy of the sampling method used,

[3] In calculating the results of the analyses of these samples, the possible presence of traces of aconitic acid has been neglected. Although the precision is excellent, the accuracy is open to slight question; that is to say, the correct curve may occupy a position a little different from that in the diagram. These points are at present under investigation.

isocitric acid does not share in the diurnal variation of acidity in *Bryophyllum calycinum* leaves.

Undetermined organic acid

The difference between the sum of the malic, citric, isocitric acids and the total organic acids is plotted in figure 1 (dotted line) as undetermined organic acid. Inasmuch as this difference contains all of the analytical errors of the separate determinations, too much weight cannot be placed upon the accuracy of the results. However, it is evident that the samples contained a small proportion of organic acids in addition to those that were directly determined. One of the components of this fraction is oxalic acid, for the presence of traces of oxalic acid in Bryophyllum leaves has been established in earlier papers from this laboratory (**6, 7**). Determinations were not made in the present series of samples, but, from previous experience, the quantity would be expected to be 20 m.eq. per kilo or less; oxalic acid therefore presumably makes up only a fraction of the undetermined organic acid shown in the figure.[4]

The trend of the curve suggests that a part of the undetermined acid is irreversibly decomposed during the culture period but there is no indication of a diurnal variation in quantity. However, further study by more sensitive analytical methods will be needed before the part played by these substances becomes clear.

Starch

Figure 2 shows the behavior of the starch. At dawn, the leaves contained 9.6 grams per kilo of starch but this had increased to 25.3 grams by early afternoon of the sunny first day and to 25.9 grams (*i.e.* by 16.3 grams per kilo) by evening. Starch formation practically ceased by 2 P.M. of this day; the additional four or five hours of bright sunlight gave rise to only a small further increase. During the night, starch dropped to 12.7 grams but increased again, although slowly, throughout the cloudy second day of the experiment reaching the level of the previous evening by sunset. The starch content changed less strikingly during the second night, but, the following day, reached the level of 26 grams per kilo by sunset and again underwent a sharp fall during the third night. It is important to note that starch attained essentially the same maximum on three successive days.

The curve in general resembles that for the total acidity, although the fluctuations are in the reverse direction. In order to illustrate this point more vividly, the total organic acids have been plotted on a suitable scale

[4] Preliminary chromatographic tests by Mr. C. W. H. Partridge of this laboratory suggest that there are nine components in the organic acid fraction of *B. calycinum* leaves of which four have been identified as oxalic, malic, citric, and isocitric acids. The other components are present in extremely small proportions; one of them is possibly succinic acid.

downward from the top of the figure (dotted line, ordinate at right of figure 2) so that the vertical mirror image of the actual organic acid curve can be directly compared with the curve for starch. The close relationship between them is obvious. As further evidence, the correlation coefficient of the total organic acid vs. starch was calculated; this gave the negative value -0.916 for the 11 cases. The probability that this result

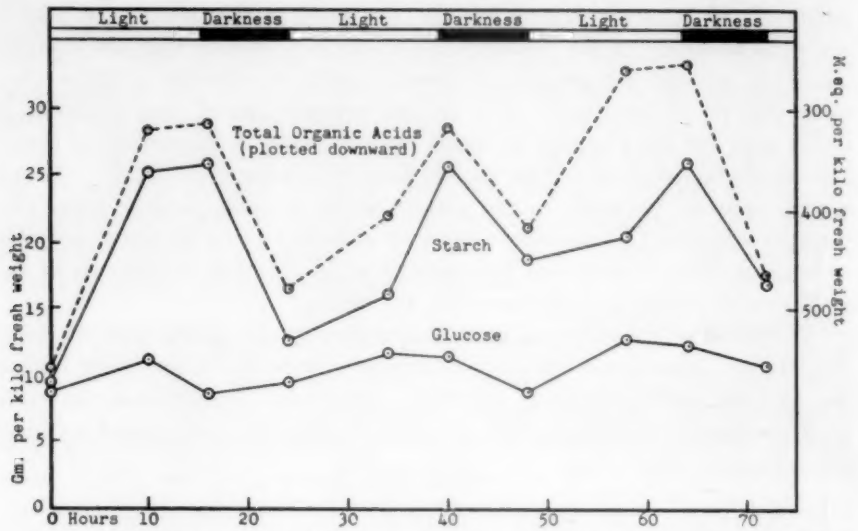

FIG. 2. Diurnal variation of starch and glucose of excised leaves of *Bryophyllum calycinum* cultured in water. Alternations of light and darkness indicated at top of figure. Data are grams per kilogram of initial fresh weight. Total organic acids plotted downward (ordinate at right) as dotted line to show close relationship between variation of acidity and that of starch.

is one of chance is less than one in a thousand ($r = 0.847$ for $P = 0.001$; 9 degrees of freedom). An even closer correlation was found during the initial 48 hours; the first 8 observations gave $r = -0.977$ ($r = 0.925$ for $P = 0.001$; 6 degrees of freedom).

A calculation of the relationship between the observations for pH and the quantities of starch gave the smaller but still highly significant correlation coefficient 0.854 for the 11 samples. Inasmuch as pH is a function that is inverse to the acidity, the correlation coefficient of pH vs. starch is positive.

The behavior of glucose (i.e. copper reducing material after hydrolysis calculated as glucose) is also shown in figure 2; the curve indicates that only small fluctuations took place. Inasmuch as hydrolysis of starch to glucose is the probable first step in the decomposition of starch, while condensation of glucose is the final step in its formation, a certain degree of regularity in the relative proportions of starch and glucose might be anticipated. However, it seems clear that mechanisms exist which maintain

the glucose concentration in the tissues at a fairly constant level in spite of the wide variations in the quantity of starch.

Discussion

The present experiment confirms and extends the result of the earlier one (**7**) and again emphasizes the close connection between the metabolism of organic acids and of starch. The evidence for an equilibrium relationship between organic acids on the one hand and starch on the other suggested that a calculation of the relative molar quantities of the reactants would be of interest. The results of such a calculation are shown in table II. The figures are offered for consideration with some reserve and

TABLE II

RELATIONSHIP OF CHANGES IN STARCH AND GLUCOSE TO CHANGES IN MALIC AND CITRIC ACIDS

	CHANGE IN GLUCOSE*	CHANGE IN MALIC AND CITRIC ACIDS†	RATIO Δ ACIDS: Δ GLUCOSE	RATIO Δ MALIC: Δ CITRIC ACID
	millimoles	*millimoles*		
First day	+101.0	−117.6	1.16	8.9
First night	−76.6	+82.1	1.07	5.5
Second day	+91.6	−58.2	0.64	11.0
Second night	−58.3	+53.4	0.92	4.7
Third day	+65.5	−81.8	1.25	4.8
Third night	−64.9	+98.2	1.51	7.5
Mean			1.09 ± 0.29	

* The change in glucose is computed from the change in starch content between the samples taken at sunrise and at sunset by multiplying by the factor 1.11; this is corrected for the change in glucose which, save during the first night, was in the same direction in all cases. The figures are millimoles per kilo of initial fresh weight.

† The change in malic and citric acid is the sum of the changes in each acid between the samples taken at sunrise and sunset expressed as millimoles per kilo of initial fresh weight.

should be regarded as merely preliminary in nature, for only analytical determinations of unusual accuracy made upon samples that duplicate each other in initial composition even more closely than the present ones could be expected to yield reliable ratios. However, the figures show, with an overall uncertainty of the order of 27 per cent., that the relative quantities are such that one mole of organic acid most of which was malic acid was converted to one mole of glucose the greater part of which accumulated as starch during each of three successive periods of daylight while the reverse change took place during the alternate periods of darkness. The energy necessary to provide for carbohydrate synthesis was obviously derived from sunlight and it would appear that a fairly high level of illumination is required if the change is to be rapid and extensive. It should further be pointed out that, although these interconversions involved quantities of the order of 15 grams of organic solids per kilo of

leaf tissue, that is to say, about 17 per cent. of the organic solids of the samples, no significant net change in the total quantity of organic solids present in the system was detected.

The relative molar quantities of malic and citric acids concerned in the successive interconversions are shown in the last column (table II); during the first day, malic and citric acids entered into reaction in the proportion of 8.9 moles of malic acid to 1 mole of citric acid; during the following night, 5.5 moles of malic acid were produced for each mole of citric acid formed, and so forth. There is little regularity in these figures and probably none could be expected unless the experiment were carried out under rigidly controlled conditions with respect to light supply, temperature, and humidity, and with samples of closely similar initial composition.

With regard to the enzymatic mechanisms whereby these interconversions were brought about, there is little to add to the views that have previously been expressed (**7**). The evidence suggests that the organic acids concerned are members of a chain of reversible enzymatic reactions which presumably represent a series of oxidation, decarboxylation and other steps whereby glucose derived from the hydrolysis of starch is alternately consumed and reproduced. A systematic series of reactions such as those which comprise the well-known Krebs tricarboxylic cycle is a possible expression of the sequence of chemical events. However, there are two observations which make it difficult to conclude that exactly this system is the one that operates in the case of diurnal variation of acidity in Bryophyllum leaves. The first is the fairly prominent part taken by citric acid in the reactions. In the tricarboxylic acid cycle as at present proposed to account for the metabolism of carbohydrate in animal tissues (**4**), citric acid is represented as being a product of a side reaction whereby *cis*-aconitic acid is in equilibrium with citric acid through the mediation of, presumably, the enzyme aconitase. *Cis*-aconitic acid occurs in the main sequence of reactions. This is not an insuperable objection in the present case but it is, nevertheless, difficult to understand.

The other objection is the fact that isocitric acid does not appear to share at all in the diurnal variation of acidity in Bryophyllum. This substance is present in large relative proportions in Bryophyllum leaves and it occurs in the main sequence of reactions of the Krebs cycle. It is difficult to see how so extensive a chemical change as that observed in the present experiment could take place, by means of reactions in which isocitric acid plays an active part, without any detectable influence upon the quantity of isocitric acid in the system.

This observation further raises the important question of the function of isocitric acid in Bryophyllum leaves. It is a curious fact that, so far

as is now known, this rare substance is present in substantial quantities only in those plant species which are characterized by the phenomenon of a broad diurnal variation in acidity. It seems, however, that isocitric acid takes no part in these changes in the particular species which provides the classical example of diurnal variation. There is an anomaly here which requires explanation.

Summary

Excised leaves of *Bryophyllum calycinum* cultured in water under greenhouse conditions for three days undergo successive alterations in organic acid and starch content in mutually opposite directions in a manner which suggests a close relationship between the metabolism of these substances. The observations thus accord with the views of Bennet-Clark. The extent and speed of the reactions appear to be influenced by the weather conditions, being appreciably greater on a bright sunny day than upon a partially cloudy day.

Malic acid is the organic acid which undergoes the greatest fluctuations in concentration, but citric acid shares in them to a moderate extent; isocitric acid, however, does not appear to vary at all.

In spite of the fact that the interconversions of starch to organic acids and the reverse involve quantities of the order of 17 per cent. of the organic solids of the tissues, no significant alteration in organic solids from sample to sample was observed. It may be inferred that the reactions which involve exchanges of gas with the atmosphere were such that there was no detectable net change in weight.

Preliminary calculations of the relative molar quantities of the substances concerned suggest that approximately one molar proportion of glucose (mostly derived from starch) is transformed to one molar proportion of malic and citric acids (malic acid the main component) during the night and that the reverse reaction occurs during the day. It is possible that the enzymatic mechanisms involved are analogous to those described by the Krebs tricarboxylic acid cycle, but the direct part taken by citric acid and the failure of isocitric acid to share at all cannot easily be accounted for by the hypothesis that the Krebs cycle, in the form widely accepted as the explanation of carbohydrate oxidation in animal tissues, exactly defines the mechanism of diurnal variation of acidity in *Bryophyllum calycinum* leaves.

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EFFECTS OF GROWTH SUBSTANCES ON PHOTOSYNTHESIS

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(WITH ONE FIGURE)

Received January 19, 1949

Introduction

The use of growth substances upon plants as herbicides and for the purpose of modifying such phenomena as dormancy, abscission, bud differentiation, and fruit set has become quite extensive. Therefore, it should be of interest to know what effect some of these compounds have upon the process of photosynthesis. Very few data have appeared in the literature in regard to this process in relation to growth substances. Mitchell, Kraus, and Whitehead (**5**) report that the leaves of bean plants which had been depleted of sugar, starch and dextrin, by being kept in the dark, accumulated less sugar, starch and dextrin during subsequent illumination when they were treated with alpha-naphthalene-acetic acid than similar untreated control plants. The authors suggest that one possible explanation of these data may be a reduction in the rate of photosynthesis.

In the present problem in which apparent photosynthesis was measured it seemed desirable to record the rates of respiration as one basis for interpretation of the results. Pratt (**6**) found that indole-3-acetic acid at 0.1 to 100 p.p.m. increased respiration of wheat seedlings but that higher concentrations of the auxin decreased respiration. Berger, Smith and Avery (**1**) concluded that indole-acetic acid at 10 mg. per liter stimulated respiration in Avena coleptiles. Zimmerman and Hitchcock (**8**) reported that naphthalene-acetic acid caused a decrease in the rate of respiration of tomato plants during the first hour after treatment after which the rate was faster in the treated plants than in the controls. Hsueh and Lou (**4**) reported that 2,4-D at a concentration of 0.1 per cent. inhibited aerobic respiration in barley and rice. The amount of oxygen used was greatly decreased as a result of the treatment. The production of CO_2 following treatment with 2,4-D was accelerated at first and then decreased but not as much as oxygen utilization. In terms of CO_2 production Brown (**2**) found that treatment with 2,4-D at 0.1 per cent. concentration resulted in a higher rate of respiration in bean plants. The results of Taylor (**7**) indicate that 2,4-D at concentrations 0.25 to 10 p.p.m. decreased respiration in wheat and mustard seedlings.

Methods

The growth substances used in this research were the acids, indole-3-acetic, gamma (indole)-butyric, beta-naphthoxyacetic, 2,4-dichlorophenoxy-

acetic, and para-chlorophenoxyacetic. Each acid was dissolved in a small amount of ethanol and then dispersed in water at a concentration of 100 p.p.m. The solutions were applied to the test plants in the form of a mist through the use of an atomizer spray.

The plants in the experiments were the common bush beans, *Phaseolus vulgaris* (var. stringless greenpod). The plants were allowed to grow in separate pots in the greenhouse until they had developed two or three compound leaves. By this time they were approximately one foot tall. The plants were selected for uniformity and arranged in pairs. One plant of each pair was sprayed with a growth substance while the others were kept for controls. Spraying was accomplished by placing the plants, one at a time, in a box, open on one side, where they were subjected to a fine mist until they were wet almost to the dripping stage. The plants were allowed to dry in the laboratory before being used. To eliminate, as far as practicable, changes in photosynthetic area during the course of the experiments all terminal and lateral buds as well as immature leaves were removed from all of the plants at the beginning of an experiment. Two matched pieces of plate glass, notched for the stem, were placed across the top of each pot and served as the bottom for a bell jar which was placed over the top of the plant. In this manner the top of each plant was enclosed in a bell jar. Photosynthesis and respiration were each determined for two-hour periods by measuring the changes in the carbon dioxide content of the air which was caused to pass through the bell jars and a series of absorption towers as previously described (**3**). Respiration was always determined immediately after photosynthesis was measured.

Data from more than 20 replicated determinations of the carbon dioxide content of air from a common source, both with and without plants, were analyzed statistically. The measurements in terms of milligrams of carbon dioxide per cubic foot of air had a standard deviation of $\pm$ .2. This is equivalent to an error of from 1–2 per cent. calculated on the basis of the carbon dioxide content of the air which often varied from day to day. The apparatus was tested for accuracy regularly throughout the series of experiments. No variation in the carbon dioxide content of air around the test and control plants was considered significant unless it was more than twice the standard deviation.

Natural illumination was used in all measurements of photosynthesis. Often it was necessary to shade the bell jars with two to four layers of cheesecloth to prevent the development of excessively high temperatures around the plants. Temperature measurements with a Leeds and Northrup thermopotentiometer and light determinations with a Weston illumination meter were made several times during the course of each experiment. Light and temperature were uniform for both the control and test plants. During the measurements of respiration the bell jars were covered with several layers of black cloth.

Results

It was found through preliminary experiments that even though two sets of plants were selected for uniformity of size, age and appearance they often exhibited quite different rates of photosynthesis and respiration per unit leaf area. To take care of such variability in the plants in each experiment the relative rates of photosynthesis and respiration were first determined for the plants which were to serve as controls and the test plants which were to be treated later. These results, listed in table I under the heading, "Before treatment," established the relative performance of the two sets of plants before any treatment with growth substances. Then after the test plants had been treated with a particular growth substance the measurements were repeated each day for a period of from two to four days. Any significant variations between the relative rates of photosynthesis and respiration in the test and control plants (T/C table I) before and after treatment of the test plants can be attributed to the treatment.

2,4-D treatment

With one exception the data from all experiments with 2,4-D exhibited the same trend. The results presented in table Ia and figure 1A are the means of two experiments. For photosynthesis the initial measurements established that the test plants, before treatment, had a rate of 0.9 that of the controls (T/C = 0.9). One day after treatment the ratio of the rate in the treated to the rate in the controls had decreased to 0.8. By the second day after treatment the treated plants seemed to have largely recovered, but through the third and fourth days their rate of apparent photosynthesis relative to the controls was 20 per cent. less than before the treatment (figure 1A). The data indicate that the principal effect of this concentration of 2,4-D on apparent photosynthesis is one of retardation.

With respect to respiration, 2,4-D after first causing a drop in the rate of respiration produced an acceleration of this process. The relative rate of respiration between the test and control plants was found to be 0.9 (T/C = 0.9) before treatment of the test plants. After treatment there was an initial decrease (table Ia, T/C = 0.7) in the ratio of respiration in the treated plants to that in the control plants. But through the second and third days after treatment the relative rate of respiration in the treated plants was from 10–20 per cent. (fig. 1B) more than it was before they were sprayed with 2,4-D. These data regarding the effect of 2,4-D on respiration in general confirm the conclusions of BROWN (**2**) and HSUEH and LOU (**4**).

Indole-butyric acid

The results presented in table Ib are the averages of three experiments in which gamma (indole-3)-n-butyric acid was used. The data from all experiments showed the same trend. The effects of this growth substance

upon photosynthesis and respiration in bean plants can be most easily observed by looking at figure 1A, B.

In regard to photosynthesis, the test plants, before treatment with indole-butyric acid, had a rate of apparent photosynthesis 1.1 times that of the plants which were to serve as controls. On two successive days following treatment the rate in the same test plants decreased to a value only 0.9 that of the controls. The experiments were only continued for two days but throughout this period the rate of apparent photosynthesis was depressed by the indole-butyric acid treatment. That this decrease in apparent photosynthesis was not due to a simultaneous acceleration in respiration may be observed from the graph in figure 1B. The data indicate that 24 hours after treatment with indole-butyric acid the rate of respiration remained unchanged with respect to the controls. But by the end of two days this growth substance had caused a decided drop in the rate of respiration in the bean plants.

Beta-naphthoxyacetic acid

The mean results of three experiments in which bean plants were treated with naphthoxyacetic acid are presented in table Ic. A comparison of relative rates of photosynthesis in the test and control plants before and after treatment of the test plants, figure 1A, leads to the conclusion that this growth substance first caused an acceleration in apparent photosynthesis. This increase persisted for three days, reaching a peak during the second day. By the end of four days the relative rate of photosynthesis in the treated and control plants was the same as before treatment.

From an examination of the graph, figure 1B, and the data in table Ic it is obvious that naphthoxyacetic acid also caused an increase in the rate of respiration for a period of two days following its application to the test plants. However, during the third and fourth days following treatment the relative rates of respiration in the test and control plants was the same as before treatment.

Indole-3-acetic acid

Following the treatment of the bean plants with indole-3-acetic acid there was a marked decrease in apparent photosynthesis, table Id and figure 1A, which persisted during the three-day period of the experiment. Before treatment the test plants had a rate of photosynthesis 1.5 times that of the control plants. After treatment of the test plants with this growth substance their rate of photosynthesis dropped until by the third day it was only 0.8 that in the control plants. The decrease in the rate of apparent photosynthesis in the treated plants cannot be attributed entirely to an acceleration of respiration in these plants. The data relative to respiration, table Id and figure 1B, indicate that although indole-3-acetic acid did result in a rise in respiratory rate two days after its application to the plants, by the third day it caused a drop in this process. In other words, by the end of three

days there was a decrease in both photosynthesis and respiration as a result of the treatment.

Para-chlorophenoxyacetic acid

Four experiments were performed with chlorophenoxyacetic acid. In general all of the results showed the same trend. The data presented in table Ie for this growth substance are the averages from two experiments

TABLE I

PHOTOSYNTHESIS AND RESPIRATION IN BEAN PLANTS BEFORE AND AFTER TREATMENT WITH GROWTH SUBSTANCES. C-PLANTS = CONTROL PLANTS WHICH WERE NOT TREATED. T-PLANTS = TEST PLANTS WHICH WERE TREATED AFTER A PRELIMINARY DETERMINATION OF THE RATES OF PHOTOSYNTHESIS AND RESPIRATION

		CO_2 EXCHANGE IN MG./DM.^{2} OF LEAF/HR.				
	TIME IN DAYS	Before treatment	After treatment			
			1	2	3	4
a. 2,4-D						
Photosynthesis:	C-plants	2.2	6.3	2.7	4.1	4.4
	T-plants	1.9	4.9	2.5	2.8	3.0
	Ratio T/C	0.9	0.8	0.9	0.7	0.7
Respiration:	C-plants	1.8	1.8	1.5	1.0	
	T-plants	1.7	1.3	1.6	1.0	
	Ratio T/C	0.9	0.7	1.1	1.0	
b. Indole-butyric acid.						
Photosynthesis:	C-plants	2.7	6.1	5.4		
	T-plants	2.9	5.4	4.8		
	Ratio T/C	1.1	0.9	0.9		
Respiration:	C-plants	1.2	1.0	1.7		
	T-plants	1.2	1.0	1.0		
	Ratio T/C	1.0	1.0	1.6		
c. Naphthoxyacetic acid.						
Photosynthesis:	C-plants	3.9	5.7	6.1	4.5	5.0
	T-plants	3.1	5.1	6.2	3.9	4.2
	Ratio T/C	0.8	0.9	1.0	0.9	0.8
Respiration:	C-plants	2.0	1.3	2.2	1.9	2.1
	T-plants	1.0	0.8	1.4	0.9	1.1
	Ratio T/C	0.5	0.6	0.6	0.5	0.5
d. Indole-acetic acid.						
Photosynthesis:	C-plants	1.6	4.4	4.0	10.0	
	T-plants	2.4	5.0	4.7	8.0	
	Ratio T/C	1.5	1.1	1.2	0.8	
Respiration:	C-plants	3.3	2.0	0.8	1.4	
	T-plants	2.6	1.5	0.8	0.8	
	Ratio T/C	0.8	0.8	1.0	0.6	
e. Chlorophenoxyacetic acid.						
Photosynthesis:	C-plants	3.5	5.4	3.4	5.8	
	T-plants	4.7	4.7	3.3	2.2	
	Ratio T/C	1.3	0.9	1.1	0.4	
Respiration:	C-plants	1.2	1.7	1.0	1.6	
	T-plants	1.3	2.5	1.4	1.5	
	Ratio T/C	1.1	1.5	1.4	0.9	

which were continued for the longest time. The relative effects may be readily observed from figure 1A, B. At the concentration used, chloro-

phenoxyacetic acid resulted in a pronounced decrease in apparent photosynthesis in the bean plants. This effect became more pronounced with time. It seems probable that part of the initial drop in apparent photosynthesis in the treated plants may be due to a simultaneous increase in respiration. For two days after treatment the rate of respiration was accelerated in the treated plants. By the third day this effect had disap-

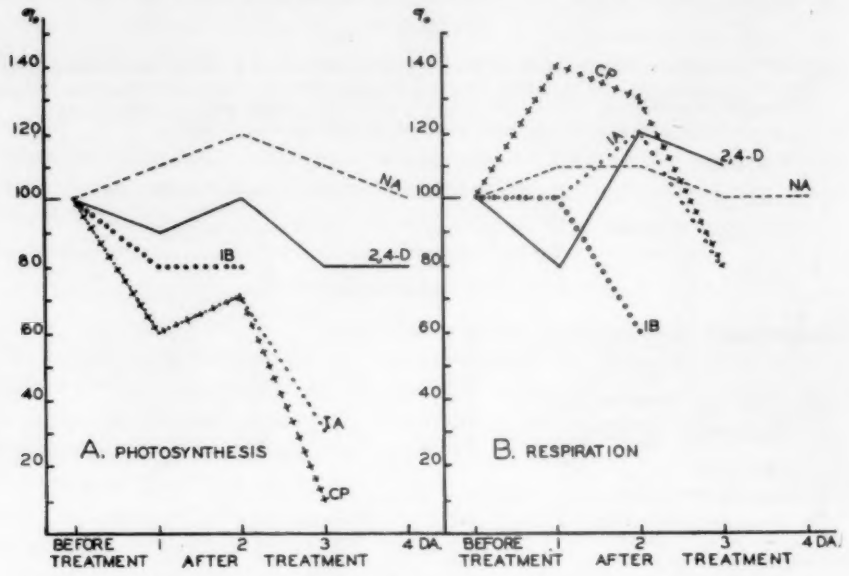

FIG. 1. Relative rates of photosynthesis and respiration in bean plants before and after treatment with growth substances when the rates before treatment are arbitrarily set at 100 per cent. of the rate in the control plants. NH, naphthoxyacetic acid; IB, indole-butyric acid; IA, indole-3-acetic acid; CP, chlorophenoxyacetic acid.

peared and there was even a 20 per cent. decrease in the respiratory rate of the treated plants relative to the controls on the basis of their comparative rates before treatment.

Discussion

In testing the effects of growth-regulating substances on the process of photosynthesis it was impossible because of time and facilities to try a large number of such compounds or many concentrations. At the start of the problem several compounds and concentrations were examined before the ones used in this paper were selected. Largely from the practical point of view it was decided to use a few of the more common growth regulators and to test their effects at a concentration of 100 p.p.m. upon carbon dioxide exchange in plants. This concentration seemed to be reasonably close to what other investigators had often found to be desirable or beneficial with respect to such phenomena as fruit set and rooting.

Of the five growth substances tested only naphthoxyacetic acid produced

an acceleration in the rate of apparent photosynthesis in beans. This beneficial effect, although quite marked for three days, did not persist and was not in evidence on the fourth day following treatment.

Apparent photosynthesis was decreased in the plants when they were sprayed with either 2,4-dichlorophenoxyacetic acid, indole-butyric acid, indole-acetic acid, or chlorophenoxyacetic acid. This effect lasted throughout the course of the experiments which ran from two to four days. The results with indole-acetic and chlorophenoxyacetic acid were much more pronounced than those from either 2,4-D or indole-butyric acid treatment. With respect to 2,4-D this seems strange in view of its well known drastic effects upon certain growth phenomena. A study of the curves in figure 1A, B discloses that the effects produced by these growth substances on apparent photosynthesis cannot be accounted for entirely on the basis of their effects upon respiration. With the exception of indole-butyric acid all of the growth substances sooner or later caused an increase in the rate of respiration. Naphthoxyacetic acid resulted in an increase in both apparent photosynthesis and respiration for two days following its application. The low rate of apparent photosynthesis for the first two days following treatment with chlorophenoxyacetic acid may be due to a corresponding acceleration of respiration but on the third day both processes were depressed. Indole-acetic acid resulted in lowered rate of apparent photosynthesis throughout the experiment but only on the second day could an accelerated rate of respiration have been partly responsible. Indole-butyric acid caused a sharp drop in respiration on the second day following its use while apparent photosynthesis was depressed from the start. On the first day after treating the plants with 2,4-D both respiration and photosynthesis were depressed. Then the rate of respiration was accelerated which may account in part for the low rate of photosynthesis after the second day.

That the growth substances used do effect the process of photosynthesis in bean plants, mostly adversely, there is no doubt. Whether the effects are direct or indirect is an unsolved problem. Preliminary investigation indicates that the effects are not explainable on the basis of the effects of the growth substances upon the opening and closing of stomates.

Summary

At a concentration of 100 p.p.m. the effects of the acids of 2,4-dichlorophenoxyacetic, indole-3-acetic, gamma (indole-3)-n-butyric, beta-naphthoxyacetic and para-chlorophenoxyacetic were determined upon apparent photosynthesis and respiration in bean plants. Each growth substance was applied as a mist spray.

With the exception of naphthoxyacetic acid all of these growth substances caused a decrease in the rate of apparent photosynthesis in beans which continued throughout the duration of the experiments which lasted from two to four days. Naphthoxyacetic acid resulted in an acceleration

of photosynthesis over a period of three days after which the rate relative to that of the controls became the same as before treatment.

In regard to respiration all of the growth substances, except indole-butyric acid, caused a temporary increase in the rate of respiration. With indole-acetic and chlorophenoxyacetic acids the initial accelerated rate of respiration was followed by a drop in the rate. The first effect of 2,4-D was a drop in respiration. Naphthoxyacetic acid caused a slight increase in carbon dioxide production for two days after which there was no acceleration. Following the treatment with indole-butyric acid the rate of respiration decreased after the first day.

The effects of the growth substances used upon the rate of apparent photosynthesis in beans cannot be accounted for entirely upon the basis of their effects upon respiration.

Acknowledgment is gratefully made to Mr. Frank Nicol whose assistance was made possible by a research grant from the Graduate School of Northwestern University.

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FREEZING INJURY OF MAIZE SEED[1]

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(WITH FIVE FIGURES)

Received May 16, 1949

Introduction

Since 1921 there have been eight years in which less than 90 per cent. of the corn crop in Iowa matured without frost damage. During the period 1921–1945 the average percentage maturing without damage was 88 per cent., with only 33 per cent. maturing safely in 1924 and 65 per cent. in 1945.

Maturity of the crop is the primary factor determining the damage done by frost. Freezing temperatures kill the foliage and stalk of immature plants, thus stopping translocation to the partially developed grain. The grain itself may be damaged with lowered keeping qualities and viability. Injury is especially serious to the hybrid seed corn industry where expensively produced seed may be reduced to low grade feed.

Freezing injury to plants has been divided into sudden killing by intracellular crystal formation, and slow desiccation and precipitation of the protoplasm when moisture is withdrawn by intercellular ice formation. Levitt (**3**) and Luyet and Gehenio (**5**) have published extensive reviews of the theories of cold injury.

Kiesselbach and Ratcliff (**2**) conducted a study of freezing injury to open-pollinated seed corn from 1913 to 1920, but open-pollinated varieties have been replaced by hybrid corn for which there are few data on frost injury. The present study was undertaken to determine the effects of temperature, moisture content of the seed, length of freezing period, variety of seed, physiological maturity, rate of freezing and thawing, repeated freezing and thawing, and rate of drying on freezing damage to hybrid maize seed. Yields of corn grown from frozen and unfrozen seed were compared.

Materials and Methods

Experiments with soaked seed

Soaked seed of U. S. 13 (WF9 × 38–11) × (L317 × Hy) was used in a preliminary investigation of the effects of moisture content, time and temperature of freezing. Moisture content was varied by mixing dry seed with measured volumes of water and holding for four days at 7° C with

[1] Journal paper no. J-1640 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project nos. 163 and 896, in cooperation with the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture.

frequent stirring. This temperature is below the minimum for growth of corn (**4**) and permitted time for equilibrium moistening of the grain without the germination changes which might have reduced freezing resistance rapidly. Samples for moisture content were taken after soaking, dried at 100° C for 24 hours, and moisture percentage calculated on a wet weight basis. Moisture content of individual kernels was checked on a chainomatic balance. It was found that, with frequent turning of the bottles during soaking, the variability in moisture content of individual kernels did not exceed ± 1.5 per cent. of the desired value.

Resoaked, dried protoplasm could hardly be expected to behave like the active protoplasm of immature seeds, and the results of these studies show important differences from those run later.

Seed for each freezing treatment was placed in four-ounce bottles and stoppered lightly with corks. These small bottles were cooled at 35° F for eight hours and then uniformly exposed in a freezing compartment which was thermostatically controlled to ± 0.3° F. The thermostat was set for the desired temperature and the appropriate bottles were removed at the end of the specified time of exposure called for by the various treatments. The samples were allowed to thaw for eight hours in the 35° F cool chamber.

The dry seed, containing 8.5 per cent. moisture, and soaked seed with 20, 25, 30, or 35 per cent. moisture was frozen for 0, 3, 8, 36, 72, or 216 hours at 20°, 15°, 10°, or 0° F. Since nine days were required to complete all of the freezing at one temperature, four separate soaking and freezing operations were necessary; one for each of the four freezing temperatures. All samples to be frozen were placed in the freezing chamber at the same time.

After thawing, the seed was planted in sand in a greenhouse bench for germination and seedling vigor tests with three replications of 50 seeds per plot. Unfrozen check samples of each moisture level were planted at the same time as each lot of frozen seed. Sixteen days after planting, counts were made for strong germination, and green weights of the seedling tops cut at the ground level were obtained.

Experiments with maturing seed

Small blocks of inbred and single cross plants were grown in the corn breeding nursery at Ames in 1946 and 1947. The inbreds were detasseled to produce F_1 seed and the single crosses were open pollinated to produce F_2 seed. In 1946 WF9 plants were pollinated by M14 in one block and Os420 was pollinated by W22 in another. Hybrid plants (WF9 × M14) were used to produce larger ears of nearly the same genetic constitution as the WF9 × M14 single-cross seed. In 1947 the two single crosses WF9 × M14 and Os420 × W22 were planted in blocks. Developing ears were harvested when the seed contained 65 to 30 per cent. moisture and were subjected to controlled laboratory freezing experiments with and without

husking. The freezing temperatures and lengths of exposures were chosen on the basis of typical fall freezes.

A check on the moisture content of the seed of all varieties in the field was maintained with frequent moisture tests. When these preliminary tests indicated that the seed was at approximately the desired moisture content, 10 to 15 ears for each freezing treatment to be made were snapped with husks attached and taken to the Botany Department low temperature rooms. Each ear was tagged to identify it throughout the procedure of freezing, moisture sampling, drying, and shelling. Ears for unfrozen checks were harvested at the same time as the samples for freezing. The ears were placed in a 35° F cool room for six to eight hours. Thermocouple readings indicated that the temperature under the husks was 35° to 37° F after six to eight hours at 35° F.

Seventy-five to 150 ears were placed on racks within the freezing chamber, which was thermostatically controlled to $\pm$ 0.3° F. The 10 to 15 ears for each treatment were randomly distributed on the racks. An electric fan was used to provide uniform air temperature within the compartment. The temperature of the freezing chamber was approximately 35° F when filled; the thermostat was then set for 26°, 20°, or 14° F depending on the freezing treatment. The air temperature within the freezing chamber dropped from 35° F to 26° F, to 20° F, or to 14° F in approximately one hour, one and a half hours, and two hours, respectively, when the chamber was filled with corn. A recording hygrothermograph kept a record of temperature and humidity during each treatment. Relative humidity was 94 to 98 per cent. during all freezes at the three temperatures. The period of freezing was recorded, beginning when the air temperature within the chamber reached the temperature specified for the particular treatment. For example, ears frozen eight hours at 20° F were in the chamber approximately nine and a half hours. Unfrozen check ears were kept in the 35° F cool room during the time of freezing and thawing.

Ears were removed from the freezing chamber to the 45° F cool room to thaw for six to eight hours. Two rows of kernels were removed from each ear for a moisture sample; the wet samples were weighed, dried in an electric oven at 98° C and the moisture percentage for each ear was determined on a wet basis. Within 24 hours after thawing the ears were placed in a seed corn drier to dry at approximately 95° F. Unfrozen check ears also were sampled individually for moisture content and were dried with the frozen samples.

Since all the varieties of corn did not reach the same moisture content in the field at the same time, it was necessary to make several freezes for each of the experiments. When an insufficient number of ears of the desired moisture content was obtained in a particular lot, another sample was harvested for the same freezing treatment. Temperature and humidity conditions were reasonably uniform in all freezes made at the same temperature and for the same length of time.

Ears with grain that contained at harvest ± 2 per cent. of the desired moisture content for each treatment, as shown by the individual moisture determinations, were shelled by hand and bulked; ears which fell outside of this range were discarded. Thus, all ears with seed that contained 48 to 52 per cent., 38 to 42 per cent., or 28 to 32 per cent. moisture at harvest for a specific freezing treatment were shelled and bulked for a 50, 40, or 30 per cent. moisture sample, respectively. These samples will be referred to, hereafter, as 50, 40, or 30 per cent. moisture samples. Four to 10 ears were bulked for each treatment at each moisture content. Most samples represented five to seven ears. Some accuracy was lost by bulking but a test indicated that the germination of the bulk sample was ± 4.0 per cent. of the mean of the individual ears.

The dry shelled seed was tested for germination and seedling vigor by planting in sand in greenhouse benches at a depth of one and one-half inches. Split-plot designs were used, with four to six replications of 25 seeds per plot. Counts for strong germination and green weights of seedlings tops cut at the ground level were obtained 16 days after planting. Germinations were reported as percentages of strong plants apparently capable of continued development. Seedling weights were taken only on plants included in the count for strong germination. Germination percentages were transformed to angles (angles = arc sine $\sqrt{\text{percentage}}$) and analyzed by analysis of variance (**9**).

KIESSELBACH and RATCLIFF (**2**) found a range of 15.2 per cent. moisture content (49.2 to 64.4 per cent.) of individual kernels on the same ear of the open-pollinated varieties used in their experiments. Individual kernels from open-pollinated ears of the inbreds and single crosses used in this study were checked for moisture content. The greatest range in moisture content was 6.24 per cent.

Experimental results

Germination percentages for the five moisture levels and six freezing times at 20° and 0° F are shown in figures 1 and 2. Germination of soaked corn frozen at 10° F was not significantly higher than that at 0° F, while that frozen at 15° F was intermediate but nearer the 0° F than the 20° F treatment.

Seed at 8.5 and at 20 per cent. moisture was not damaged by any of the treatments. Seed containing 25 per cent. moisture showed no reduction in germination from exposures at 20° and 15° F, but the germination was reduced as much as 33 per cent. by exposure to 10° and 0° F. Viability of seed soaked to 30 and 35 per cent. moisture was reduced severely at all temperatures and reached 0.0 per cent. with exposures of 36 hours at 10° F and 35 per cent. moisture. These results show that the resistance of the seed to freezing temperatures varied with moisture content.

The major reductions in germination occurred during the first eight hours of freezing with some further decrease up to 36 hours (figures 1 and

2). Leveling off of the curves after 36 hours indicates that time was not important with longer exposures (36 to 216 hours). The ability of some seed in the 30 and 35 per cent. moisture samples to withstand long exposures to freezing temperature may have been due either to genetic differences

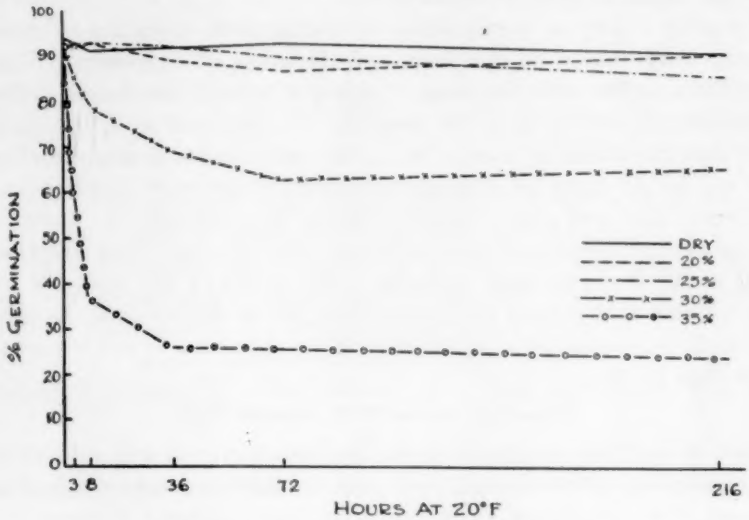

FIG. 1. Germination percentage of soaked seed frozen at 20° F.

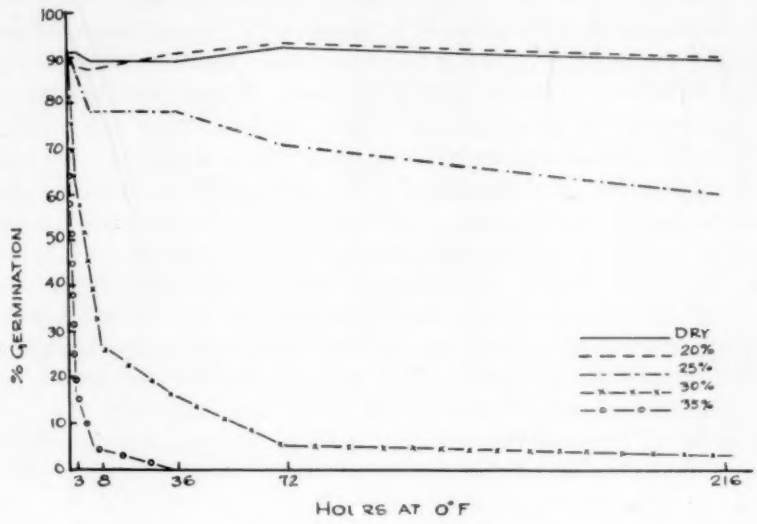

FIG. 2. Germination percentage of soaked seed frozen at 0° F.

in the seed within these samples, or to differences in moisture content. Since the tests showed uniformity of moistening, it appears more likely that survival was due to genetic differences among individual kernels in the open-pollinated seed from the double-cross hybrid U. S. 13 plants.

Mean germinations for all moisture levels at temperatures of 20°, 15°, 10°, and 0° F were 79.0, 72.0, 64.6, and 61.8 per cent., respectively. The effect of temperature apparently was not linear. LUYET and GEHENIO (5) state that the velocity of crystallization and the growth of ice crystals become less rapid at lower temperatures.

Freezing injury to germination of soaked seed depended on moisture content, temperature of freezing, and duration of exposure. With the soil at freezing temperatures these data might indicate the degree of damage to viability of seed corn in the ground. KIESSELBACH and RATCLIFF (2) found that when air-dry corn with 7.7 per cent. moisture was embedded in moist soil for 24 hours the embryo contained 21.4 per cent. moisture and the endosperm, 15.6 per cent. After 48 hours in moist soil, the embryo had 36.8 per cent. moisture and the endosperm, 20.8 per cent. Soil temperatures as low as 20° F are unlikely to occur in the spring of the year, but the results indicate that soaked seed containing 30 to 35 per cent. moisture is susceptible to freezing and may be injured by exposures to soil temperatures higher than 20° F.

SEEDLING VIGOR WITH SOAKED SEED

Rate of seedling emergence in the germination tests was related to the moisture content of the soaked seed, with no difference between frozen and unfrozen lots. Seedlings from the more moist samples emerged one to three days before those from the drier samples, and, as a consequence, the seedling weights were heavier at the time of harvest, 16 days after planting.

There was no injurious effect of freezing on the average weight of normal seedlings. Percentages of weak seedlings were counted, and there was no difference between unfrozen and frozen seed. Freezing injury to soaked seed apparently was an all or none effect; the seed either was killed completely or it survived and its seedling vigor was unimpaired. Ice crystals formed on the outside of the soaked seed containing 25 per cent. or more of moisture, and ice was obivous within the kernels at the higher moisture. In spite of such freezing, the grain was sometimes uninjured, or if it showed any injury it was killed. These results suggest that intercellular ice did no damage, but that the formation of intracellular ice crystals resulted in the death of the seed. Since intermediate degrees of injury were not observed, it is assumed that intracellular ice, once started, was able to spread throughout the embryo, perhaps inoculating adjoining cells through the plasmodesma. This reaction contrasts sharply with that of maturing seed, reported below, in which graded injury was obtained, suggesting more localization of areas of internal ice formation.

DISTRIBUTION OF MOISTURE IN SOAKED AND IMMATURE SEED

Distribution of moisture in the various kernel parts of soaked seed containing 20, 25, 30, or 35 per cent. moisture and of freshly harvested seed containing 35 per cent. moisture is given in table I. Moisture contents of

TABLE I

DISTRIBUTION OF MOISTURE IN VARIOUS KERNEL PARTS OF SOAKED AND FRESHLY HARVESTED SEED FROM OPEN-POLLINATED PLANTS OF U. S. 13

KERNEL PART	FRESHLY HARVESTED SEED	SOAKED SEED			
Pericarp	52.63	47.50	38.34	27.92	16.47
Embryo	48.16	54.27	47.31	33.49	21.68
Endosperm	30.71	28.41	25.68	22.69	19.38
Whole Kernel	36.01	35.38	30.69	25.46	21.15

the embryo and pericarp were consistently higher than that of the endosperm. The embryo of soaked seed was 6.11 per cent. higher in moisture than freshly harvested seed when the whole kernel of both contained 35 to 36 per cent. moisture, suggesting that soaked seed might be more severely injured by freezing than fresh seed at the same moisture level.

Soaked and freshly harvested seed at 35 per cent. moisture was frozen as indicated in table II. Shelled seed, both fresh and soaked, was frozen

TABLE II

MEAN GERMINATION PERCENTAGE FOR OPEN-POLLINATED SEED OF U. S. 13 FROZEN AT 20° F WITH VARIOUS TREATMENTS AND 35 PER CENT. MOISTURE

HOURS EXPOSURE TO 20° F	TREATMENT			
	WITH HUSKS ON EARS	WITHOUT HUSKS ON EARS	SHELLED, FRESHLY HARVESTED	SHELLED, SOAKED SEED
0	94	96	93	97
2	92	93	85	61
4	93	94	65	48
8	91	82	54	40
16	84	65	24	26

in small stoppered bottles. Seed resoaked with water after drying was more severely injured by freezing than shelled, freshly harvested seed. Higher moisture content in the embryo of soaked seed may have been a factor in the increased damage. Freshly soaked protoplasm not fully hydrated may have been more susceptible to internal ice crystal formation than the fresh protoplasm. Water may be bound more closely or more intimately mixed with the protoplasm during maturation and development of the freshly harvested seed, thereby resisting crystallization. Ice crystals formed on the outside and within the soaked kernels; no crystals were observed on the outside of freshly harvested seed but ice was observed within some of this seed.

Kernels embedded on the ear without benefit of husk protection apparently were protected by heat conduction from the cob and by placement

on the cob. The husk was an additional protector of viability at both the eight- and 16-hour exposures.

Copper-constantan thermocouples and a portable potentiometer were used to check the temperature under the husk of four inbred lines during freezing (table III). The temperature under the husk was approximately

TABLE III

AVERAGE TEMPERATURE BENEATH THE HUSKS OF EARS OF INBRED LINES OF CORN AT VARIOUS INTERVALS AFTER PLACING IN THE FREEZING CHAMBER AT 20° F

INBRED LINE	HOURS AFTER ENTERING CHAMBER AT 20° F						AIR TEMP.
	0	1	2	3	4	9	
	TEMPERATURE BENEATH THE HUSKS ° F						
M14	34.5	32.0	29.5	26.5	26.0	24.5	20.0
WF9	34.5	33.5	30.5	29.0	27.5	26.0	20.0
Os420	35.0	33.5	29.5	27.5	27.0	26.5	20.0
W22	35.0	32.5	30.5	28.0	27.0	25.5	20.0
Mean	34.8	32.9	30.0	27.8	26.9	25.5	20.0

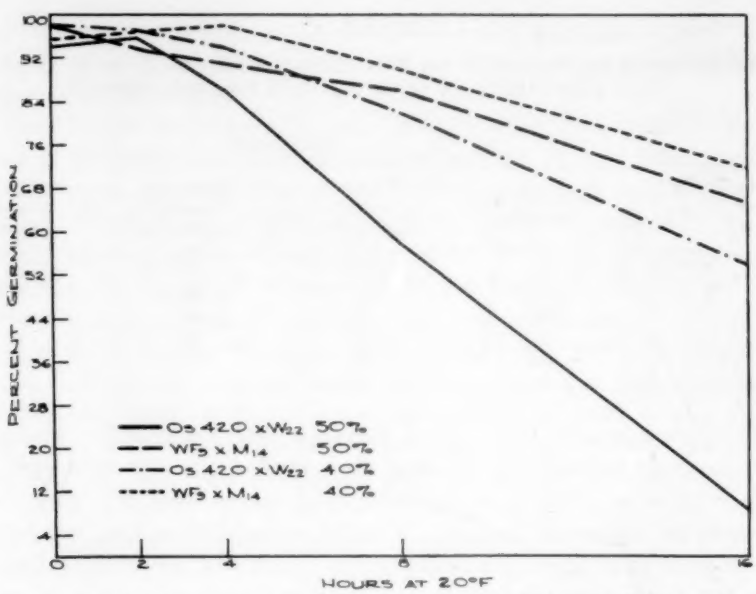

FIG. 3. Germination percentage of F_1 seed of WF9 × M14 and Os420 × W22 frozen at 20° F. 1946.

35° F when the ears were placed in the freezing chambers. Air temperature of the chamber dropped from 35° to 20° F in one and one-half hours. The average temperature under the husk was 25.5° F after nine hours in the chamber. These results indicate that the husk may effectively reduce freezing injury by slowing heat losses.

TABLE IV

MEAN GERMINATION PERCENTAGE FOR F_1 SEED OF WF9 × M14 AND Os420 × W22 HARVESTED AT 50, 40, OR 30 PER CENT. MOISTURE AND FROZEN 0, 2, 4, 8, OR 16 HOURS AT 14, 20, OR 26° F. 1946

DURATION OF FREEZING (HOURS)	TEMPERATURE OF FREEZING												GRAND MEAN
	14° F				20° F				26° F				
	MOISTURE CONTENT OF SEED			MEAN	MOISTURE CONTENT OF SEED			MEAN	MOISTURE CONTENT OF SEED			MEAN	
	50%	40%	30%		50%	40%	30%		50%	40%	30%		
	F_1 SEED OF WF9 × M14												
0	100.0	97.0	98.0	98.3	98.0	95.0	95.0	96.0	96 0	99.0	97.0	97.3	97.2
2	96.0	98.0	97.0	97.0	94 0	97.0	100.0	97.0	99.0	98.0	97.0	98.0	97.3
4	71.0	82.0	96.0	83.0	91.0	98.0	96.0	95.0	98.0	98.0	99.0	98.3	92.1
8	22.0	64.0	91.0	59.0	86 0	90.0	100.0	92.0	97.0	100.0	100.0	99.0	83.3
16	1.0	4.0	68.0	24.3	65.0	72.0	97.0	78.0	93.0	95.0	99.0	95.7	66.0
Mean	58.0	69.0	90.0		86.8	90.4	97.6		96.6	98.0	98.4		
				72.3				91.6				97.7	87.2
	F_1 SEED OF Os420 × W22												
0	95.0	100.0	96.0	97.0	94.0	98.0	96 0	96.0	97.0	98.0	97.0	97.3	96.8
2	80.0	91.0	99.0	90.0	95 0	97.0	97.0	96.3	98.0	95.0	96.0	96.3	94.2
4	44.0	62.0	90.0	65.3	86.0	94.0	97.0	92.3	97.0	94.0	100.0	97.0	84.9
8	13.0	22.0	86.0	40.3	58.0	82.0	98.0	79.3	93.0	98.0	100.0	97.0	72.2
16	0.0	1.0	55.0	18.7	9.0	50.0	98.0	52.3	95.0	97.0	100.0	97.3	56.1
Mean	46.4	55.2	85.2		68.4	84.2	97.2		96.0	96.4	98.6		
				62.3				83.3				97.0	80.8
Grand mean	52.2	62.1	87.6		77.6	87.3	97.4		96.3	97.2	98.5		
				67.3				87.4				97.3	84.0

Effects of temperature, freezing period, moisture content and variety on freezing injury

Mean germination percentages for the 90 treatments involving F_1 seed of WF9 × M14 and Os420 × W22 are given in table IV. Germination percentages for 20° and 14° F freezing are plotted in figures 3 and 4.

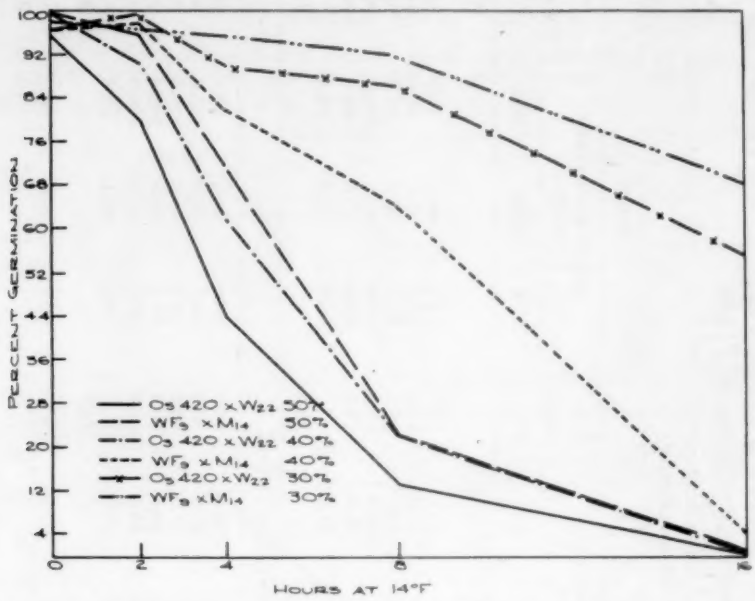

Fig. 4. Germination percentage of F_1 seed of WF9 × M14 and Os420 × W22 frozen at 14° F. 1946.

Where conditions were severe enough to produce injury, seed of WF9 × M14 was consistently less damaged than seed of Os420 × W22. The difference between the two varieties was dependent on the severity of the freezing and the moisture content. These varieties do not represent the extremes in varietal differences that exist in corn when the seed is at a similar moisture content (**8**).

Exposures up to 16 hours at 26° F resulted in no injury to either variety. Temperatures of 20° F and 14° F significantly reduced viability of both varieties. Exposures of two or four hours at 20° F produced no injury to either variety. When subjected to eight or 16 hours at 20° F viability was reduced if the seed contained 50 or 40 per cent. moisture. Seed containing 50 or 40 per cent. moisture was injured by exposures of four to 16 hours at 14° F. That containing 30 per cent. moisture was uninjured in all treatments except the eight and 16 hour freeze at 14° F. Seed with 50 or 40 per cent. moisture was damaged by 20° or 14° F depending on the length of exposure. Two-hour exposures at either of the temperatures did not reduce germination.

Decreasing the temperature from 26° to 20° F reduced the germination

9.9 per cent. as an average for all moistures, durations, and varieties. A further decrease in temperature from 20° to 14° F gave an average of 20.1 per cent. reduction. With the temperatures used here, there was no indication of a leveling-off in the effect of temperature as was apparent with the soaked seed frozen at 10° and 0° F and described above, probably because the protection of ears and husks prevented injury with the less severe treatments.

The main effects, temperature, moisture content, and duration of exposure, and the first and higher order interactions were significant when tested against the random error term (7). All first and second order interactions involving varieties were either highly significant or significant, showing that the effects of temperature, duration of exposure, and moisture content differed with the variety. Figures 3 and 4 show the presence of these interactions and emphasize the difficulty in making accurate statements about the general effect of freezing conditions on seed viability. These results demonstrate the necessity of knowing the specific details of variety and moisture content of the seed, temperature of freezing, and length of exposure before one can estimate the damage that might occur. Other factors that may also influence injury will be brought out in later sections of this study.

Germination percentages for F_2 seed of WF9 × M14 frozen in 1946 are presented in table V. No highly significant injury occurred when seed containing 40 per cent. moisture was frozen at any of the three temperatures. Seed with 55 and 65 per cent. moisture was injured significantly by eight-hour exposures at 20° F, while that with 50 and 40 per cent. moisture was apparently safe from damage. At 14° F seed with 50, 55, or 65 per cent. moisture was damaged by treatments of four and eight hours, but uninjured by a two-hour exposure. Two and four hours of 20° F temperature produced no injury.

A temperature of 26° F was not fatal to any of the seed. The temperature underneath the husk of ears from inbred lines given an eight-hour freeze at 20° F did not go below 25.5° F (table III), and it is probable that seed on ears exposed to 26° F air temperature was not injured because the temperature under the husk did not reach the freezing point of the grain.

F_2 seed of WF9 × M14 was injured less by an eight-hour freeze at 14° F than the F_1 seed of WF9 × M14 (table IV). Germinations were 44.0 and 22.0 per cent. at 50 per cent. moisture and 90.4 and 64.0 per cent. at 40 per cent. moisture for the two lots. Ears of several double-cross hybrids were frozen eight hours at 20° F in 1946 when the seed contained 40 per cent. moisture. Viability was not reduced in any of the seed. Probably the larger ear size of the single- and double-cross plants was a factor in delaying cooling and thereby reducing the freezing injury to the seed.

Mean germination percentages for the F_2 seed of WF9 × M14 and Os420

TABLE V

MEAN GERMINATION PERCENTAGE FOR F_2 SEED OF WF9 × M14 HARVESTED AT 65, 55, 50, OR 40 PER CENT. MOISTURE, FROZEN 0, 2, 4, OR 8 HOURS WITH HUSKS ON EARS AT 14, 20, OR 26° F. 1946

DURATION OF FREEZING (HOURS)	TEMPERATURE OF FREEZING															GRAND MEAN
	14° F					20° F					26° F					
	MOISTURE CONTENT OF SEED				MEAN	MOISTURE CONTENT OF SEED				MEAN	MOISTURE CONTENT OF SEED				MEAN	
	65%	55%	50%	40%		65%	55%	50%	40%		65%	55%	50%	40%		
0	76.0	88.0	94.4	96.8	88.8	74.4	88.0	92.8	95.2	87.6	75.2	87.2	92.0	97.6	88.0	88.1
2	66.4	85.6	92.8	97.6	86.0	71.2	91.2	92.0	97.6	88.0	75.2	89.6	92.8	98.4	89.0	87.7
4	49.6	70.4	79.2	95.2	73.6	76.0	86.4	91.2	96.0	88.2	73.6	94.4	95.2	98.4	90.4	84.1
8	19.2	35.2	44.0	90.4	47.2	58.4	66.4	85.6	96.0	76.6	66.4	92.0	90.4	96.8	86.4	70.1
Mean	52.8	70.2	77.6	95.0	73.9	70.0	83.0	90.4	97.0	85.1	72.6	90.8	92.6	97.8	88.5	82.5

TABLE VI

MEAN GERMINATION PERCENTAGE FOR F_2 SEED OF WF9 × M14 AND Os420 × W22 HARVESTED AT 50, 40, OR 30 PER CENT. MOISTURE, AND FROZEN 0, 4, 8, 16, OR 24 HOURS WITH HUSKS ON THE EARS AT 14, 20, OR 26° F. 1947

DURATION OF FREEZING (HOURS)	TEMPERATURE OF FREEZING												GRAND MEAN
	14° F				20° F				26° F				
	MOISTURE CONTENT OF SEED			MEAN	MOISTURE CONTENT OF SEED			MEAN	MOISTURE CONTENT OF SEED			MEAN	
	50%	40%	30%		50%	40%	30%		50%	40%	30%		
	F_2 SEED OF WF9 × W14												
0	90.0	96.0	99.0	95.0	91.0	95.0	97.0	94.3	89.0	95.0	95.0	93.0	94.1
4	60.0	79.0	91.0	76.7	82.0	88.0	99.0	89.7	87.0	97.0	93.0	92.3	86.2
8	2.0	29.0	76.0	35.7	68.0	80.0	92.0	80.0	78.0	93.0	95.0	88.7	68.1
16	0.0	2.0	40.0	14.0	25.0	53.0	76.0	51.3	69.0	77.0	92.0	79.3	48.2
24	0.0	1.0	15.0	5.3	0.0	37.0	62.0	33.0	52.0	82.0	98.0	77.3	38.6
Mean	30.4	41.4	64.2		53.2	70.6	85.2		75.0	88.8	94.6		
				45.3				69.7				86.1	67.0
	F_2 SEED OF Os420 × W22												
0	94.0	97.0	94.0	95.0	90.0	96.0	100.0	95.3	91.0	94.0	95.0	93.3	94.6
4	31.0	60.0	88.0	59.7	69.0	89.0	92.0	83.3	73.0	98.0	98.0	89.7	77.6
8	3.0	11.0	37.0	17.0	51.0	67.0	88.0	68.7	59.0	87.0	95.0	80.3	55.3
16	0.0	4.0	6.0	3.3	2.0	39.0	55.0	32.0	51.0	84.0	99.0	78.0	37.8
24	0.0	0.0	0.0	0.0	0.0	17.0	44.0	20.3	35.0	83.0	90.0	69.3	29.9
Mean	25.6	34.4	45.0		42.4	61.6	75.8		61.8	89.2	95.4		
				35.0				59.9				82.1	59.0
Grand mean	28.0	37.9	54.6		47.8	66.1	80.5		68.4	89.0	95.0		
				40.2				64.8				84.1	63.0

× W22 frozen in the autumn of 1947 are shown in table VI. The analysis of variance (**7**) showed the presence of significant interactions involving temperature, duration of freezing, moisture content, and variety. These interactions again emphasize the importance of knowledge of the specific combination of these factors before making decisions on the freezing injury that might occur to seed corn.

F_2 seed of WF9 × M14 was more tolerant than comparable seed of Os420 × W22. Reductions in viability occurred with various exposures at all three temperatures. Seed with 50 per cent. moisture was, in general, injured by all exposures to 26°, 20°, or 14° F. The one exception was seed from WF9 × M14 exposed for four hours at 26° F; in a comparable treatment, seed from Os420 × W22 was injured. Loss of viability in seed with 40 per cent. moisture started with a 16-hour exposure at 26° F, eight hours at 20° F, and four hours at 14° F. Damage to the germination of seed containing 30 per cent. moisture was confined to temperatures of 20° and 14° F.

A comparison of tables V and VI shows that injury was consistently greater in the dry season of 1947 than in 1946, when similar freezing treatments were made on the same variety of seed at the same moisture content.

Seedling vigor with immature seed

Mean green weight per seedling top, cut at the ground level, was obtained for the freezing treatments on F_1 seed of WF9 × M14 and Os420 × W22 in 1946. Only those plants classified as normal, strong seedlings were included in the weighings. In contrast to the results with soaked seed, seedling vigor was significantly reduced by the more severe freezing treatments. Where viability of the seed was seriously reduced, vigor of the seedlings also tended to be impaired. Reductions in seedling vigor of frosted seed were also noted in the immature material treated in 1947. There did not appear to be any consistent difference in rate of emergence between frosted and unfrosted seed. In general, seedlings from the more mature seed emerged a day earlier than those from less mature seed. Conditions for germination were favorable and the seedlings emerged five or six days after planting in sand.

Weak seedlings were counted in all experiments when final germination counts were made. Percentages of weak seedlings were greater in treatments showing more severe freezing injury than in the unfrozen checks or in treatments with relatively little injury. Some seedlings were unable to emerge through the sand, and were not included in the counts for weak seedlings. In some cases the coleoptile emerged but no leaves were produced; other seedlings developed one or two leaves and ceased further development. Seedlings were found with radicles but no plumules, and some with plumules but no radicles.

The associations of reductions in seedling weight and increases in weak

seedlings with a loss of viability on freezing suggest that the factors responsible for death were associated with a loss in vigor. The exact mechanism cannot be determined from these data. Freezing injury was apparently not an all or none effect as when soaked seed was frozen. Seeds were capable of various levels of germination and seedling growth, indicating that the degree or extent of injury to the protoplasm differed among the seeds. Reduction in seedling weight of normal-appearing plants may have been more a result of injury to the endosperm, and increases in abnormal seedlings may have resulted from injury to the embryo. In all seeds examined, ice was present in seed containing 50 to 40 per cent. moisture and frozen eight to 24 hours at 20° or 14° F. It was difficult to detect ice in seed with 30 per cent. moisture. Germination tests showed that not all of the seed was killed even though ice was present. The progressive type of injury, from reduced vigor to complete death of the seed, suggests an effect of intercellular ice formation with gradual desiccation and irreversible coagulation of protoplasm. Abnormalities in which certain seedling parts failed to develop suggest localized killing in the embryo, perhaps by intracellular ice formation which failed to spread throughout the seed as it seemed to do with soaked seed. Any attempts to observe the process of freezing within the seed and to differentiate between inter- and intracellular crystallization would require examination of cut sections, which cannot be expected to provide a true picture of what actually happens in the whole seed during freezing.

Yield of grain from frozen and unfrozen seed corn

A yield test was conducted in 1947 to determine if the reductions in seedling vigor would influence yields of grain in the crop grown from frosted and immature seed. Single-cross seed of W22 × WF9, Os420 × W22, WF9 × Os420, and WF9 × W22 was used. Ears were harvested in 1946 when the seed contained 50, 40, or 30 per cent. moisture and were given three freezing treatments: (A) frozen eight hours at 20° F with husks on the ears; (B) frozen eight hours at 20° F without husks on the ears; and (C) unfrozen check. Germination percentages were obtained from tests conducted in sand. There were nine treatments for each of the four single crosses, making 36 treatments in the experiment. The yield test was planted at Ames in a split-plot design with four replications of single row plots ten hills long. Sufficient seed (up to ten kernels per hill in the low germination treatments) was planted to assure a uniform stand. All plots were thinned to three plants per hill. Drought conditions prevailed during the latter part of the growing season and yields were low.

Plants from the less mature seed were not as vigorous as those from mature seed during the early part of the season, but no difference in vigor was apparent at tasseling time. There were no significant differences in yield for any of the treatments. Moisture content and freezing treatment

of the seed at harvest time produced no significant effects on yield of grain in the resulting crop when approximately equal stands were obtained.

Seed maturity

Percentage of moisture or dry matter in the grain is generally recognized as the standard measurement of maturity in corn, although a better measurement of maturity might be the attainment of maximum dry matter content per kernel. The term "seed maturity" as used in this study refers to the percentage of total dry matter accumulation in the kernel, and is intended to indicate relative physiological maturity of the seed. Oven-dry weights of seed harvested at 65 to 40 per cent. moisture were divided by the oven-dry weight of seed harvested at 30 per cent. moisture. With increasing dry matter, protoplasmic changes occur which may favor frost resistance. Properties of protoplasm that may be associated with increasing dry matter content are: higher cell sap concentration, more "bound" water, and increased quantities of non-aqueous but plastic substances such as protein, fat, oil, and starch. Thus, protoplasm may be more viscous and resist irreversible dehydration and cell contraction during freezing.

Dry weight per 50 kernels and percentage seed maturity are presented for F_1 seed of WF9 × M14 and Os420 × W22, for F_2 seed of WF9 × M14 in 1946, and for F_2 seed of WF9 × M14 and Os420 × W22 in 1947 (table VII).

The two varieties of F_1 and F_2 seed differed significantly in seed size, but apparently the larger seed size of Os420 × W22 was not an advantage since it was injured more by freezing than WF9 × M14. F_2 seed was similar in appearance and shape to F_1 seed produced on inbred plants of WF9 and Os420. The two varieties responded in the same order of tolerance to freezing temperature whether the seed was F_1 seed produced on inbred parents or F_2 produced on F_1 plants. Both types of WF9 × M14 seed reached 50, 40, or 30 per cent. moisture three to four days before Os420 × W22, while the data of pollination or silking was approximately the same for the two varieties.

There was no significant difference in seed maturity of the F_1 seed of the two varieties, indicating that the varietal difference in response to freezing temperature was due to other factors. Seed produced on F_1 plants of the two varieties in 1947 showed a highly significant difference in seed maturity. Seed produced on Os420 × W22 plants had a higher percentage of total dry matter accumulated (greater maturity) at 50 or 40 per cent. moisture, yet it was significantly more injured by freezing than WF9 × M14. In a study of relative tolerance to freezing temperature of a group of inbred lines, however, a highly significant correlation was found between seed maturity and tolerance (**8**). The more mature inbreds, as measured by percentage of total dry matter accumulated in the seed, tended to be more tolerant to freezing temperature. The lack of agreement in the two experiments suggests that factors other than dry matter accumulation

and moisture content may be important in tolerance of the seed of different varieties to the same freezing conditions.

Freezing injury to F_2 seed of WF9 × M14 was more severe in 1947 than in 1946 (tables V and VI). A possible explanation for this difference can be obtained from table VII. Seed produced in 1947 was less mature than

TABLE VII

MEAN DRY WEIGHT PER 50 KERNELS AND MEAN MATURITY

MOISTURE CONTENT OF SEED %	DRY WEIGHT IN GRAMS		SEED MATURITY PER CENT.	
	WF9 × M14	Os420 × W22	WF9 × M14	Os420 × W22
		F_1 SEED IN 1946		
50	10.2	13.1	81.9	83.3
40	11.6	15.1	93.6	95.4
30	12.4	15.8	100.0	100.0
		F_2 SEED IN 1946		
65	5.7		46.2	
55	8.1		65.9	
50	9.1		74.3	
40	10.9		89.3	
30	12.2		100.0	
		F_2 SEED IN 1947		
50	7.6	9.1	66.7	71.2
40	9.5	11.5	83.7	90.0
30	11.4	12.8	100.0	100.0

in 1946 at the same moisture content, as indicated by the lower percentage of total dry matter accumulated at 50 and 40 per cent. moisture. Further evidence is shown in table VIII, which gives the mean seedling weights

TABLE VIII

MEAN GREEN WEIGHT OF TOP IN GRAMS PER SEEDLING GROWN FROM UNFROZEN F_2 SEED OF WF9 × M14 HARVESTED AT 50, 40, OR 30 PER CENT. MOISTURE IN 1946 OR 1947

% MOISTURE CONTENT AT HARVEST	YEAR SEED WAS PRODUCED		1947 / 1946
	WEIGHT IN GRAMS 1946	WEIGHT IN GRAMS 1947	
50	.55[1]	.38	69.1
40	.84	.55	65.5
30	.92	.75	81.5
Mean	.77	.56	72.7

[1] Least significant difference between any two means in body of table
= .04 grams at 5 per cent. level
.06 grams at 1 per cent. level

from unfrozen seed harvested at 50, 40, or 30 per cent. moisture in the two years. For this comparison seed from both seasons was planted in the greenhouse in sand at the same time. Seedling vigor of 1947 seed was significantly less than the vigor of 1946 seed. The very dry season of 1947 was conducive to more rapid drying of the seed in the field, so that although the seed contained the same moisture content as in 1946 it was actually at a less mature stage of development and more susceptible to the same freezing conditions. Seed with 30 per cent. moisture in 1947 was only 7.1 per cent. lighter in weight than 1946 seed, but there was 18.5 per cent. difference in seedling weight, indicating that the quality as well as the quantity of endosperm reserves may have differed in the two years.

Kernel development at the same moisture percentage may differ, depending on weather conditions and on the particular variety involved. While moisture content of the seed is an important factor influencing the degree of freezing injury, the stage of kernel development and probably other factors not apparent in these results are also important.

A study was made of the distribution of moisture and dry matter content in various ear and kernel parts of F_2 seed of Os420 × W22 and WF9 × M14 in 1947. Ears of Os420 × W22 had more husk protection (table IX)

TABLE IX

MOISTURE AND DRY MATTER CONTENT OF VARIOUS PARTS OF EARS FROM PLANTS OF WF9 × M14 AND Os420 × W22. 1947

		WF9 × M14	Os420 × W22
Green weight of ear plus husk	gm.	305	352
Green weight of husk	gm.	29	59
Dry weight of husk	gm.	11	20
Moisture content of husk	%	62.0	66.1
Green weight of cob	gm.	98	86
Dry weight of cob	gm.	30	31
Moisture content of cob	%	69.4	64.0
Green weight of grain	gm.	184	203
Dry weight of grain	gm.	92	98
Moisture content of grain	%	50.0	51.7
Number of kernels per ear	approx.	590	508
Dry weight per kernel	gm.	.1558	.1928
Dry weight of husk per kernel	gm.	.0187	.0393
Dry weight of husk/dry weight of grain	gm.	.1195	.2041

as measured by green and dry weight of husk, by dry weight of husk per kernel, and by dry weight of husk/dry weight of grain, yet Os420 × W22 ears were more susceptible to freezing injury. Both varieties had 10 to 12 husk leaves per ear, so the difference cannot be explained by the number of air layers protecting the seed. No temperature readings under the husk were obtained on these two varieties, but data obtained on inbred lines (table III) showed no appreciable difference in temperature under the husk of inbred lines that differed in freezing tolerance (**8**).

Compared with WF9 × M14 (table X) the kernel of Os420 × W22 was larger, the pericarp was greater by weight and by percentage, the embryo was larger and contained a slightly higher moisture content when the grain was at 50 per cent. moisture. The thicker pericarp of Os420 × W22 seed was apparently not an effective barrier against freezing injury.

TABLE X

MOISTURE CONTENT, DRY WEIGHT, AND PERCENTAGE OF VARIOUS KERNEL PARTS OF F_2 SEED OF WF9 × M14 AND Os420 × W22 AT TWO STAGES OF MATURITY. 1947

	Entire kernel	Pericarp	Embryo	Endosperm
Moisture content %				
(1) Os420 × W22	50.18	64.00	60.57	44.98
(2) WF9 × M14	49.50	68.26	59.26	44.55
(3) Os420 × W22	35.56	50.57	49.65	30.79
(4) WF9 × M14	36.87	52.29	48.86	31.30
Dry weight in grams				
(1) Os420 × W22	.1862	.0214	.0204	.1444
(2) WF9 × M14	.1580	.0116	.0157	.1307
(3) Os420 × W22	.2424	.0254	.0335	.1835
(4) WF9 × M14	.1959	.0123	.0238	.1598
Percentage dry weight				
(1) Os420 × W22	100.00	11.51	10.95	77.54
(2) WF9 × M14	100.00	7.34	9.93	82.73
(3) Os420 × W22	100.00	10.50	13.86	75.64
(4) WF9 × M14	100.00	6.28	12.15	81.57

On the contrary, it may have provided a larger area for more rapid ice crystallization, since the pericarp was much higher in moisture content than the endosperm. Greater susceptibility of Os420 × W22 may have been due to a less favorable size and moisture relationship of embryo to endosperm, but the data do not warrant conclusions. It appears that the problem of varietal differences in frost injury is complex, but the results suggest that properties of the protoplasm enable some varieties to withstand freezing better than others.

Rate of freezing and thawing

LEVITT (**3**) reviewed the literature in which the rate of freezing and the rate of thawing plant tissues have been investigated. Several cases were reported where a sudden drop in temperature increased the frost injury. Intracellular ice formation was thought to occur following strong undercooling, thus increasing the injury. The majority of investigations on the influence of the rate of thawing have failed to show an effect on frost injury. In most cases where an effect was found, artificial conditions were used and the rates of thawing were more rapid than are likely to occur in nature.

A study was made to determine if rate of freezing and thawing affect

the degree of injury to seed corn. Open-pollinated ears of Os420 × W22 were frozen for eight hours at 20° F in the husks when the seed contained 55, 50, or 45 per cent. moisture in 1947. A relatively fast rate of freezing was attained with ears cooled to 35° F before freezing, and a slower rate of freezing was obtained with ears warmed to room temperature before freezing. All ears were in the freezing chamber nine and a half hours, since it required approximately one and a half hours for the air temperature to reach 20° F. Slow thawing consisted of allowing the ears to thaw in a 45° F cool room for eight hours. Fast thawing consisted of three treatments: one lot was allowed to thaw at room temperature (approximately 75° F), another lot was thawed in the sun at 80° F, and another lot was thawed in warm water at 110° F. A sample of unfrozen ears was placed in the warm water to determine if the water affected the germination.

Germination results are presented in table XI. Slow freezing gave a

TABLE XI

MEAN GERMINATION PERCENTAGE FOR F_2 SEED OF Os420 × W22 HARVESTED AT 55, 50, OR 45 PER CENT. MOISTURE, AND FROZEN EIGHT HOURS AT 20° F, WITH HUSKS ON EARS, AT SLOW OR FAST RATES OF FREEZING AND WITH SLOW OR FAST RATES OF THAWING. 1947

Moisture %	Slow freeze		Fast freeze				Unfrozen check in warm water	Mean
	75° F Fast thaw	45° F Slow thaw	75° F Fast thaw	45° F Slow thaw	Warm water thaw	Thawed in sun		
55	53.0	56.0	25.0	26.0	25.0	26.0	89.0	42.9
50	63.0	67.0	48.0	45.0	46.0	51.0	92.0	58.9
45	79.0	81.0	64.0	67.0	62.0	66.0	97.0	73.7
Mean	65.0	68.0	45.7	46.0	44.3	47.7	92.7	

significantly higher germination than rapid freezing. The difference is believed to be due to the fact that seed on ears with slow freezing was actually exposed to a shorter period of freezing temperature. The ears contained more heat when they entered the freezing chamber and tended to remain longer at a temperature above freezing.

Rate of thawing had no significant effect on the degree of injury. The temperatures and conditions used in thawing are believed to represent a wide enough range to indicate that germination of frosted seed corn is not affected by rates of thawing likely to occur in the field after a freeze.

Repeated freezing and thawing

The first killing frost of autumn may be followed by freezes on succeeding nights. In 1947, an experiment was conducted to determine the effect of repeated freezing and thawing on the viability of seed corn.

Open-pollinated ears of Os420 × W22 were harvested at 55, 50, and 45

per cent. moisture and frozen at 20° F with husks on the ears. Six treatments were made: 24 hours continuous freezing, eight-hour freezes on three succeeding days, four-hour freezes on six succeeding days, one freeze of eight hours, one freeze of four hours, and an unfrozen check. When the ears were not in the freezing chamber they were kept in the 45° F cool room with a relative humidity of 90 per cent. or higher during the six days required to complete the freezing operations. Moisture samples for all treatments were taken on individual ears after the last treatment was completed. Germination percentages are given in table XII.

TABLE XII

MEAN GERMINATION PERCENTAGE FOR F_2 SEED OF Os420 × W22 HARVESTED AT 55, 50, OR 45 PER CENT. MOISTURE, AND GIVEN VARIOUS FREEZING TREATMENTS AT 20° F. 1947

MOISTURE %	TREATMENT						MEAN
	24 HRS. CONTINUOUS	8 HRS. 3 TIMES	8 HRS. ONCE	4 HRS. 6 TIMES	4 HRS. ONCE	UNFROZEN CHECK	
55	0.0	6.0	33.0	49.0	59.0	93.0	40.0
50	0.0	12.0	50.0	57.0	73.0	95.0	47.8
45	2.0	53.0	72.0	73.0	81.0	97.0	63.0
Mean	0.7	23.7	51.7	59.7	71.0	95.0	

A continuous 24-hour exposure to 20° F was significantly more injurious to viability than repeated exposures of eight and four hours. With shorter periods, the seed under the husk was undoubtedly subjected to a shorter total exposure to 20° F because of the lag between air temperature and seed temperature. Repeated freezes of eight and four hours gave a highly significant reduction in germination, but the total effect of the first freeze was not repeated on each subsequent freeze. For instance, one freeze of eight hours at 45 per cent. moisture reduced the germination 25 per cent., while two additional freezes produced a further decrease of only 24 per cent. Some drying may have occurred after the first freeze, thereby reducing the injury on the following freezes. These data emphasize the importance of duration of freezing temperatures.

RATE OF DRYING AFTER FREEZING

After a premature freeze in the field, the seed producer often is eager to harvest seed corn because he fears another freeze may occur and further injure the crop. The purpose of this study was to determine if frozen seed corn might recover somewhat from freezing injury if allowed to dry slowly in the field instead of being dried quickly in the drier soon after freezing.

The enzymes of a frozen tissue tend to be less injured than the more complex protoplasmic structures (**1**). If some of the surviving enzymes

effect protoplasmic repairs or continue normal ripening processes in the injured but still partially viable grain, seed quality might be expected to improve during slow drying.

Open-pollinated ears from single-cross plants of Os420 × W22 were harvested in 1947 at 55, 50, or 45 per cent. moisture, and frozen eight hours at 20° F with husks on the ears. Moisture content of the grain of each ear was determined from a sample cut from the tip of the ear. One group of ears was placed in a seed-corn dried within 12 hours after thawing; another group was allowed to dry slowly with husks attached to the ears in front of an open window in a shed. Six to eight ears within ± 1 per cent. of the desired moisture were shelled by hand and bulked for each treatment. Germination tests were made in sand approximately two months after freezing.

Slow drying of frozen seed resulted in a higher germination percentage for the three moisture contents (table XIII). The mean increase was 20.3

TABLE XIII

MEAN GERMINATION PERCENTAGE FOR F_2 SEED OF Os420 × W22 HARVESTED AT 55, 50, OR 45 PER CENT. MOISTURE, FROZEN EIGHT HOURS AT 20° F, WITH HUSKS ON THE EARS, AND DRIED AT SLOW OR FAST RATES. 1947

MOISTURE %	DRYING RATE		MEAN
	FAST	SLOW	
55	35.0	52.0	43.5
50	44.0	68.0	56.0
45	59.0	79.0	69.0
Mean	46.0	66.3	

per cent., a highly significant difference. The protoplasm of the seed is undoubtedly in a very sensitive condition after freezing and its subsequent treatment may determine whether or not it survives. During freezing, the protoplasm is dehydrated as a result of ice formation; another quick dehydration of the protoplasm soon after thawing by drying in a seed-corn drier may result in fatal injury to some seeds that might survive with slower drying. Other workers (**3**) have reported that injury to frosted plant tissue may increase or decrease depending on conditions after thawing.

A freeze severe enough to injure the germination of seed corn would be certain to kill the foliage of the plant. Further translocation of food material to the seed would cease and the seed would dry slowly if allowed to remain in the field. The seed-corn producer might improve the germination of his frosted seed by leaving the crop in the field for some time after freezing, providing still more severe freezing was not encountered. Early killing frosts are frequently followed by periods of warmer weather in which considerable recovery of viability could occur.

Effect on dormancy

Sprague (**10**) reported that immature seed in the fresh condition was slow and irregular in germination until the moisture content of the seed was reduced to 25 per cent. or lower. Attempts to break the dormant condition of fresh grain by soaking in sodium thiocyanate or hydrogen peroxide were ineffective. Removal of the pericarp and storage under increased oxygen pressure were also ineffective unless a loss in moisture occurred. In contrast, premature sprouting of kernels on ears of corn in the field may be rather prevalent during the autumn.

A study was undertaken to determine if freezing temperature has an effect on the dormant condition of freshly harvested immature seed. Open-pollinated ears from WF9 × M14 plants were harvested at approximately 40

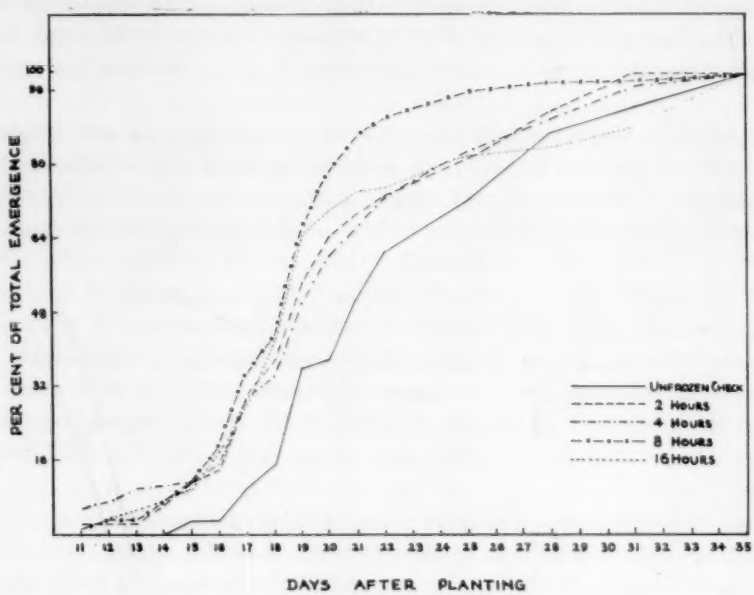

Fig. 5. Percentage of total emergence at various days after planting (one day after freezing).

per cent. moisture, and frozen 0, 2, 4, 8, or 16 hours at 20° F. Moisture content of the seed of each ear was determined and ears with 40 ± 2 per cent. moisture were shelled carefully by hand and bulked for each treatment. Shelled seed was spread in a thin layer after freezing and allowed to dry at room temperature until planted. A sample of the seed of each treatment was planted in sand with four replications of 25 seeds per plot one, three, five, seven, and nine days after freezing. Seedling emergence was counted daily on all plots.

Percentages of total emergence for the five treatments planted one day after freezing when the seed contained approximately 40 per cent. moisture are shown in figure 5. Frosted seed emerged more rapidly than unfrosted.

Some of the premature sprouting of kernels on the ear may result from freezing temperature. Similar results were obtained for seed planted three days after freezing at approximately 35 per cent. moisture. When the seed had dried to approximately 20, 11, and 9 per cent. moisture (five, seven, and nine days after freezing) there was no appreciable difference in rate of emergence between frozen and unfrozen seed.

Discussion

The exact mechanism of freezing injury to seed corn cannot be determined from the results of this study, but the data obtained suggest that both intercellular and intracellular ice formation are concerned. Any attempt to observe the locale of crystallization would necessitate microscopic examination of cut sections, which is an unnatural condition, and would not be expected to give a true picture of what actually happens in the whole kernel during freezing. Detailed microscopic studies after freezing may provide clues.

Presence of ice in the seed was not an indication that the seed was killed, since some of the seed in which ice could be observed was capable of strong germination. Frozen seed was usually spongy and soft at the higher moisture content, and was very susceptible to rapid infection unless exposed to favorable drying conditions soon after freezing and thawing. After drying in the seed-corn drier, severely frozen seed showed a darkened color of the embryo which agrees with the previous work (**2**), but some of the samples of frozen seed with low germination percentages were indistinguishable from comparable unfrozen seed on the basis of embryo color. After further drying at 98° C for dry weight determinations the endosperm and embryo of all the low germination samples were much darker in color than the unfrozen.

Shelled, soaked seed was more severely injured than shelled seed in the freshly harvested condition at 35 per cent. moisture (table II). Physiology of the seed differs on the two sides of the after-ripening process. In the fresh condition enzymes are relatively inactive and synthesis of protoplasm predominates. When the seed is soaked after drying, enzymes are activated and digestion of protoplasm commences. The physiological role of water is apparently different, as suggested by SPRAGUE (**10**), who found that seed corn in the freshly harvested condition was slow and irregular in germination, while the same amount of water in the seed after drying resulted in quick and regular germination. Water in the soaked seed was apparently more subject to crystallization and the protoplasm was more readily injured than in the fresh condition. Higher moisture content in the embryo of soaked seed may also have been a factor increasing the injury. Quicker killing, no reduction in seedling vigor, and no increase in percentage of weak seedlings when soaked seed was frozen suggests that death may have resulted from rapid intracellular ice

formation. In contrast to these results, shelled seed in the fresh condition was more slowly killed by freezing, suggesting a closer association of water with the protoplasm and more resistance to crystallization. Fresh seed on the cob without husks was protected by heat conduction from the cob, while ears with husks were further protected from heat losses during freezing.

Maturing ears with and without husks attached were given various freezing treatments when the seed contained 65 to 30 per cent. moisture. Several effects were noted: reduction in seedling vigor, abnormal seedlings, and finally complete death of the seed. The progressive type of injury observed suggests that intercellular ice formation occurred, accompanied by dehydration of the protoplasm and eventually an irreversible physicochemical change of the protoplasm. Localized killing, as evidenced by the failure of some frozen seed to develop either a plumule, a radicle, or more than one or two leaves suggests that localized intracellular ice formation may have occurred in the embryo. Reduction in seedling vigor may not carry over into the yield of grain grown from frozen seed, since there were no significant differences between frozen and unfrozen seed in the yields of corn produced when approximately equal stands were obtained in a poor growing season. These results agree with those obtained with immature seed (**6**).

In the experiments in which temperature, duration, moisture content, and variety of seed were investigated, it was found that all main effects and interactions were significant when tested against random error. Thus the response of any single variable was dependent upon the level of the other variables. Loss of germination as a result of freezing was dependent upon the four factors listed above and no distinction as to their relative importance can be made. Injury was directly related to the moisture content and duration of exposure and inversely related to temperature. The behavior of the two varieties of seed was similar in that WF9 × M14 was consistently more tolerant of freezing temperature than Os420 × W22 whether the seed was F_1 or F_2. Larger ear size of single-cross plants was effective in reducing the freezing injury when compared with the smaller ears of inbred plants. Seed viability was reduced considerably more in the 1947 freezes than in 1946, and the apparent yearly interaction adds to the difficulty of estimating injury.

Physiological maturity was measured by the percentage of total dry matter accumulated. Seed at 30 per cent. moisture was assumed to be physiologically mature and the dry weight of this seed was used in determining the percentage maturity at higher moisture contents. Robinson (**6**) and others have found that maximum kernel weight may be reached when the grain contains approximately 40 per cent. moisture. This figure is too high for the material used in the present study, since dry weights increased for all varieties of seed below 40 per cent. moisture.

With a higher percentage of total dry matter accumulated at a specific moisture content, the water may be "bound" more closely to the protoplasm and less subject to crystallization. The protoplasm may be more viscous and elastic, thereby resisting dehydration and reducing the amount of cell shrinkage during freezing, or the protoplasm may be chemically different.

Seed produced in 1947 was physiologically less mature than the 1946 seed at the same moisture content, which may explain the more severe injury that occurred in the 1947 freezing. Unusually hot dry weather with considerable wind during August and September of 1947 was conducive to more rapid loss of moisture from the seed in the field. While the seed harvested for freezing treatments in both seasons contained the same moisture content, the 1947 seed was actually at a less mature stage of development and more susceptible to injury.

Rapid drying after freezing was found to have a highly significant, injurious effect. When frozen seed was allowed to dry slowly after freezing, germination averaged 20.3 per cent. higher than corresponding seed dried in the seed-corn drier shortly after freezing. Protoplasm of the seed is undoubtedly very sensitive after freezing and may recover somewhat by slow drying, whereas another quick dehydration of the photoplasm soon after freezing may prevent any recovery and cause further injury. All of the frozen seed in the other experiments of this study was placed in the drier within 24 hours after freezing and the germinations reported might be lower than would have occurred if the seed had been dried more slowly.

KIESSELBACH and RATCLIFF (**2**) obtained more extensive freezing injury to seed corn, but the majority of their controlled freezing experiments were with sections of ears frozen 24 hours without husk protection. They made some controlled treatments with whole ears protected by the husks, and generally found less damage than occurred with similar treatments in the present investigation. Different varieties and more variability in moisture content of individual kernels on the open-pollinated varieties used in their work may account for some of the discrepancies.

Summary

Preliminary studies were conducted with air-dry shelled corn soaked to various moisture contents and given different freezing treatments. Maturing ears of corn with seed containing 65 to 30 per cent. moisture were harvested and frozen under controlled laboratory conditions intended to simulate typical freezes as they might occur in the field. The effects of freezing temperature on seed viability and vigor were investigated.

(1) Viability of soaked seed was injured more than seed of the same moisture content in a freshly harvested condition. Seedling vigor of soaked seed was not reduced and there was no increased percentage of weak seedlings as a result of freezing. All of the injured seeds were killed

outright. Fresh seed was protected by the cob and husk, but was still more tolerant than soaked seed under the same exposure conditions.

(2) In freezing experiments with maturing ears of corn, reduction of seed viability depended on the following factors: (a) temperature, (b) duration of exposure, (c) moisture content of the seed, (d) variety of seed, (e) husk protection, (f) physiological maturity of the seed, and (g) rate of drying after freezing.

(3) Freezing injury was greater in 1947 than in 1946. Seed corn in 1947 was physiologically less mature than the same seed in 1946 at a similar moisture content.

(4) Rate of thawing had no significant effect on the viability of frozen seed corn.

(5) Repeated freezing and thawing was less injurious than continuous freezing when the total exposure time was the same. The first exposure was more injurious than subsequent exposures to the same freezing conditions.

(6) Green weight of seedling tops from frozen, immature seed was generally reduced and there was a larger percentage of weak seedlings than in unfrozen seed. There was no significant difference between the yield of corn grown from frozen and unfrozen seed when approximately equal stands were obtained.

(7) Germination of frozen seed was more rapid than of unfrozen seed before drying.

The author wishes to express his appreciation to Drs. W. E. Loomis and G. F. Sprague for their assistance and suggestions during the course of this investigation.

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PHOTOPERIODIC CHLOROSIS IN TOMATO[1]

ALICE P. WITHROW and ROBERT B. WITHROW

(WITH THREE FIGURES)

Received April 11, 1949

Introduction

The leaves of certain plants have been shown to contain less chlorophyll when subjected to continuous artificial radiation than when given shorter photoperiods. GUTHRIE (**3**) reported that tomato, transferred from the greenhouse to continuous artificial radiation, after a period of several days developed an interveinal chlorosis and a yellowing of the leaves with a decrease in chlorophyll and a consistent lowering of the a/b ratio as compared to plants transferred to an 18-hour photoperiod. There also was a decrease in total carotenoids and in the carotene to xanthophyll ratio. With the decrease in chlorophylls, there was an increase in brown pigments, which he postulated as an indication that the chlorophyll decrease was due to decomposition rather than to decreased synthesis. ARTHUR and HARVILL (**2**) did further work on plant growth under continuous irradiation, using sodium lamps and combinations of sodium and mercury arcs. They irradiated geranium, cotton, buckwheat and other species. They found that with geranium and cotton, continuous irradiation from the sodium lamp brought about a marked chlorosis, especially of the younger leaves developed under the sodium irradiation. When the plants were given two hours daily of irradiation from a mercury vapor lamp to replace the sodium for this length of time, the chlorosis disappeared. More rapidly growing plants as buckwheat did not develop the chlorosis very markedly.

ROODENBURG (**4**) reports an interveinal chlorosis in tomato resulting when daylight was supplemented with eight hours irradiation from neon lamps to lengthen the photoperiod. ÅBERG (**1**) also reports chlorosis in leaves of tomato plants grown under continuous neon irradiation. Neither Åberg nor Roodenburg presents chlorophyll data and neither appears to have secured completely chlorotic leaves as were observed by the Boyce Thompson workers, but the interveinal chlorosis described by them appears to be similar to that observed under continuous artificial irradiation by Guthrie and Arthur and Harvill.

The present investigation was undertaken to ascertain further aspects of the photoperiodic chlorosis in tomato and the interrelationship of photoperiod and temperature on the chlorophyll content. It was also desired to

[1] Journal Paper No. 390, Purdue University Agricultural Experiment Station, Lafayette, Indiana.

determine whether this reaction has any characteristics in common with other photoperiodic responses in higher plants.

Procedure

Indiana Baltimore tomato was used throughout the investigation. The plants were seeded in subirrigation gravel culture in constant-condition rooms. The nutrient solution used consisted of the following salts given in millimolar concentrations: KNO_3, 10; K_2SO_4, 5; $CaSO_4$, 8; $MgSO_4$, 4; $Ca(H_2PO_4)_2$, 2. The solution was applied three times daily. Twice weekly a micronutrient supplement of 1 p.p.m. of Fe as $FeSO_4$ and 0.3 p.p.m. of Mn as $MnSO_4$ was added. Other essential micronutrients had been found previously in adequate supply in the gravel and the fertilizer salts used for the macronutrients. The pH was adjusted to six daily. The solution was changed at weekly intervals throughout the investigation.

The indicated temperature was held to within 1° C. Incandescent lamp irradiation, filtered through 10 cm. of water to remove the near infrared, was used for all treatments except for the irradiances of 4 and 20 fc in which case the lamps were unfiltered. For chlorophyll data, one cm^2 leaf punchings were taken from the youngest mature leaves close to the center of the leaf and including the midrib. Twenty punchings were used per sample and weighed immediately. They then were extracted in a Waring blendor for five minutes in 80 per cent. acetone. The acetone extract was filtered through a Büchner funnel with two thicknesses of Whatman No. 1 filter paper. The sample was made up to 100 ml. with 80 per cent. acetone and placed in a 125 ml. flask, corked, sealed with tape, and stored immediately in the dark at 4° C. All samples were taken in triplicate. The absorption coefficients of the extracts were obtained within 48 hours after extraction with the Beckman spectrophotometer, in some cases at intervals from 380 to 680 mμ and in others at 660 mμ when only relative chlorophyll concentrations were desired. The extent of the chlorosis was determined in relation to the following three variables: (1) length of photoperiod, (2) irradiance, and (3) temperature.

Length of Photoperiod

The plants were transplanted 14 days from seeding to the plots for variable treatment. The temperature was held at 20° C. The plants received 325 fc for 15, 18, 21, and 24 hours per day respectively and the radiation variables were begun at the time of transplanting. Prior to this, the plants had been kept in a 15-hour photoperiod at 325 fc. The plants were photographed for figure 1 after 23 days of treatment and the samples for chlorphyll determination taken on the same day.

Irradiance

The plants were seeded, transplanted, the irradiation variables begun and harvest for chlorophyll determination made at the same time as in the

above experiment. The radiation variables consisted of 15 hours per day at 325 fc supplemented with the following four conditions for the remaining nine hours: 0, 4, 20, and 325 fc respectively.

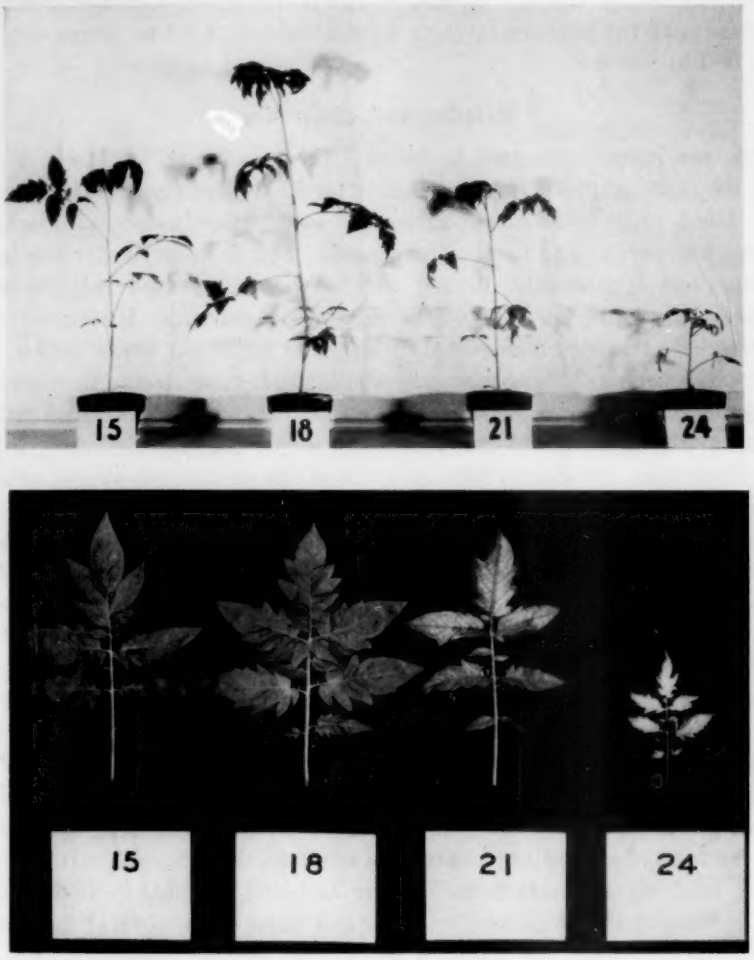

FIG. 1. Above: Response of Indiana Baltimore tomato plants to length of photoperiod. Incandescent sources at 325 footcandles were employed with the temperature controlled at 20° C and photoperiods as indicated.

Below: Leaves of tomato become chlorotic in photoperiods longer than 18 hours at a temperature of 20° C.

TEMPERATURE

The plants were transplanted to the plots for variable treatments 20 days from seeding. The irradiation and temperature variables were begun 31 days from seeding and harvest for chlorophyll samples was made after 12 days of treatment, 43 days from seeding. Prior to the application of the treatments, the temperature was 18° C, and the irradiation approximately

500 fc with a photoperiod of 15 hours. The four variable treatments included the following conditions of photoperiod and temperature with an irradiance of 325 fc in all cases: (1) 15 hours at 12° C, (2) 15 hours at 24° C, (3) 24 hours at 12° C, and (4) 24 hours at 24° C. The absorption coefficients of the acetone extracts were obtained at 10 to 20 mμ intervals from 380 to 680 mμ.

Results and discussion

The results are presented in figure 1 through figure 3. It can be seen from the photographs in figure 1 that, after 23 days of treatment, the plants were tallest under an 18-hour photoperiod and markedly decreased in height as the photoperiod was further increased. The decrease under the longer photoperiods is probably directly related to a decreased rate of photosynthesis due to the lowering of the chlorophyll content. Under a 21-hour photoperiod, the leaves acquired interveinal chlorotic areas which later developed a brown flecking markedly similar to that occurring in manganese deficiency. A 24-hour photoperiod caused the new leaves frequently to become almost entirely chlorotic, and they were small and definitely thicker than those from the shorter photoperiods as shown by area-weight relationships.

Because of the similarity of the response to certain mineral deficiency symptoms, in one experiment the leaves of plants under a 24-hour photoperiod were sprayed with a water-polyvinyl alcohol solution of the following micronutrient salts at a pH of 5: 0.1 per cent. ferrous ammonium citrate, 0.05 per cent. manganese sulphate, 0.5 per cent. magnesium sulphate, and water alone as a control. In addition a complete micronutrient spray was used consisting of 0.1 per cent. ferrous ammonium citrate, 0.05 per cent. manganese sulphate, 0.05 per cent. boric acid, 0.005 per cent. copper sulphate, 0.01 per cent. zinc sulphate, and 0.001 per cent. molybdenum trioxide. After ten days of treatment with the above solutions, none of the applications had caused any observable effect on the chlorosis. In addition to this, analysis of leaves from 15-hour and 24-hour photoperiods showed little difference in manganese content and there were several indications that in the very chlorotic leaves, a manganese content considerably higher than in the green leaves obtained.

The chlorosis was incipient at about four long photoperiods and quite noticeable by seven long photoperiods. After 14 to 21 long photoperiods, the youngest leaves were often almost entirely chlorotic, and the marbled pattern had disappeared. The older leaves which had developed under 15-hour photoperiods before transfer never showed the marked chlorotic symptoms evidenced by the younger leaves developing under the 21-hour or 24-hour photoperiods.

One pattern of development observed several times was the apparent recovery of the plant while still subjected to a long photoperiod. For three

or four weeks, the young developing leaves were very chlorotic and remained so. However, the leaves developed later than this entirely lacked

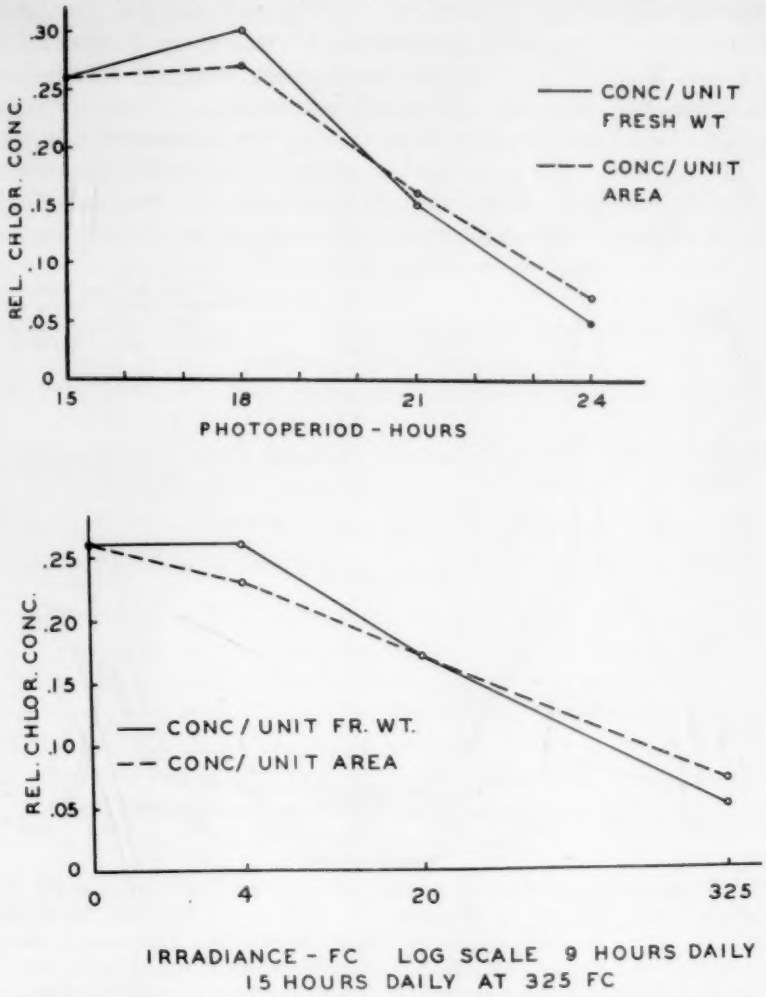

FIG. 2. Above: Relative chlorophyll concentration of acetone extracts of tomato leaves of plants grown under varying photoperiods of incandescent irradiation. A marked decrease in concentration occurs in photoperiods longer than 18 hours. Data taken at 660 mμ.

Below: Relative chlorophyll concentration of acetone extracts from leaves of plants grown under 15-hour periods at 325 footcandles of incandescent irradiation supplemented for nine hours with 0, 4, 20 and 325 footcandles to achieve a 24-hour photoperiod. A decrease in concentration based on unit area is observed at the lowest supplemental irradiance used.

the chlorosis, although the older chlorotic leaves already present did not recover. At first this was thought to be due to the higher irradiance incident to the plants as they grew closer to the lamps, but in an experiment

where an irradiance of 1500 fc was used, young plants developed the same typical chlorosis under 24-hour photoperiods as previously evidenced at 325 fc.

The relative chlorophyll concentrations of the acetone extracts for various photoperiods are shown in the upper graph of figure 2. The curves indicate that the maximum chlorophyll concentration occurred under 18 hours, a marked decrease under 21 and a very low concentration in the 24-hour photoperiod. The trend of the graph is similar whether the data are plotted on a fresh weight or unit area basis, the greatest difference occurring in the 18-hour photoperiod. The relative chlorophyll concentra-

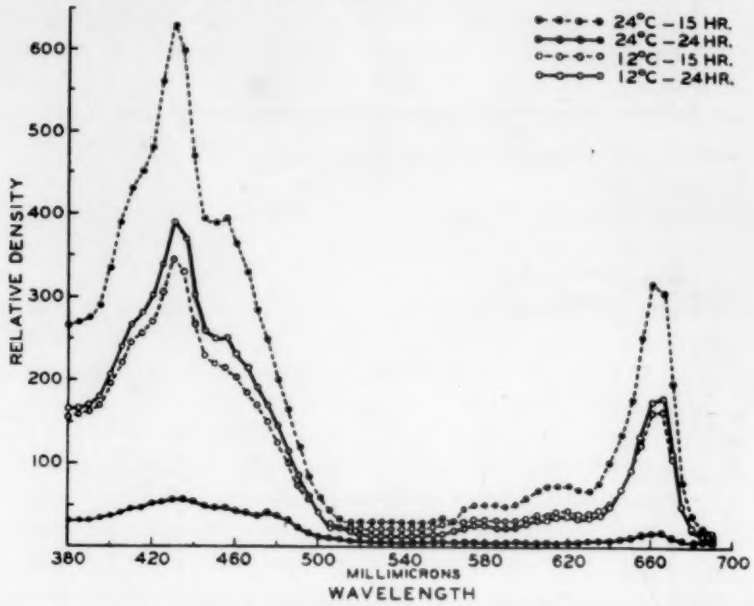

FIG. 3. Absorption spectrum of acetone extracts from plants grown under 15-hour and 24-hour photoperiods and at 12 and 24° C. Data were taken from 380 to 680 mμ as indicated. At the low temperature, there was relatively little difference in concentration of extracts from plants grown under the two photoperiods, but at 24° C, the difference was very great.

tion, on a unit area basis with the irradiance plotted on a log scale, shows a decrease with a 24-hour photoperiod as compared with a 15, even when a relatively low irradiance of 4 fc was used for nine hours to supplement a 15-hour period of 325 fc. On a unit fresh weight basis, no difference in chlorophyll concentration was shown when this low irradiance was used, although a definite mottling of the leaves was present. When an irradiance of 20 fc was used for the nine-hour period, there was a marked drop in chlorophyll concentration and a still further decrease at 325 fc.

The data presented in figure 3 on the interrelationship of temperature and photoperiod indicate that at a temperature of 12° C, photoperiod has

little effect on chlorophyll concentration. At 24° C there was a very great difference in pigment content between the 15-hour and 24-hour photoperiods. The highest concentration occurred in the 15-hour photoperiod at 24° C, with a very marked decrease in the photoperiods with the 12° C temperature and a very low concentration in the 24-hour photoperiod at 24° C. Very little mottling was observed in the plants held at 12° C although the leaves were the pale yellowish green characteristic of tomato plants held at a low temperature.

The results of this series of investigations on the effect of radiation and temperature variables on chlorophyll concentration in the leaves of tomato show several points of similarity to the effect of similar variables on the flowering of plants. First, occurrence of the chlorosis is controlled by the length of the photoperiod. It further occurs in response to a relatively low irradiance applied as a supplement to a short high irradiance photoperiod. In addition, a definite interrelationship between photoperiod and temperature on the development of the chlorosis occurs, with a failure of the chlorosis to appear at relatively low temperatures. Further investigations are underway on wavelength and other radiation variables on the synthesis and decomposition of chlorophyll in relation to photoperiod.

Conclusions

A typical chlorosis in the leaves of tomato appears at photoperiods longer than 18 hours when incandescent lamps are used as the sole source of radiant energy. This chlorosis occurs at 20° C when a 15-hour photoperiod is lengthened to 24 hours with relatively low irradiances of 4 and 20 fc. When the plants are grown at temperatures of 12° C, the length of the photoperiod has relatively little effect on the chlorophyll concentration of the leaves and the typical severe chlorotic pattern developed at higher temperatures under 24-hour photoperiods is absent.

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INFLUENCE OF POTASSIUM AND BORON ON NUTRIENT-ELEMENT BALANCE IN AND GROWTH OF RANGER ALFALFA[1]

ARTHUR WALLACE AND FIRMAN E. BEAR

Received January 15, 1949

Inadequate supplies of K and B have been associated with short life and low yield of alfalfa in the eastern United States. For this reason, studies of the influence of these two elements on the behavior of this plant are of special interest. This paper is concerned with such studies.

Literature review

HUNTER, TOTH, and BEAR (**9**) concluded that when the K content of alfalfa falls below 1 per cent., its Ca exceeds 2 per cent., or its Ca-K equivalent ratio is greater than four, yields are greatly depressed. It was also shown that although the content of the individual cations may vary widely, cation-equivalent summation values tend to be constant under standardized environmental conditions. Such cation interrelationships in plants have received considerable attention (**7, 19, 28**).

Other work from this station (**29**) has shown that plants having high cation-equivalent values also tend to have high mineral anion-equivalent values. When alfalfa plants were grown under uniform environmental conditions, except as to the nutrient supply, cation-anion equivalent ratios were essentially constant. This applied, notwithstanding variations in the relative amounts of the different cations in the nutrient medium and regardless of the season of the year. Other workers have noted this phenomenon (**17, 23**).

Extensive data on K-B and Ca-B relationships in plants are highly contradictory (**5, 18, 24**). A reciprocal relationship exists between K and Ca in alfalfa, and in other plants as well. Unavoidable variations in absorbed K, when Ca is varied, have a marked influence on Ca-B relationships. That such influences may be operative was pointed out by SHEAR *et al.* (**23**) who expressed cation-B relationships in terms of the ratios of $\frac{Ca+K}{Mg}$, $\frac{Ca+Mg}{K}$, and $\frac{K+Mg}{Ca}$ to B. BECKENBACH (**2**) showed that B requirements of tomatoes varied with P and N levels of the nutrient medium. The evidence suggests that B effects are related to all the cation and anion values in the plant. The same reasoning applies to the interrelationships among all the other nutrient elements.

[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University—the State University of New Jersey—Department of Soils, New Brunswick, New Jersey.

Methods

Two series of plants were grown, employing the constant-drip procedures of solution renewal. In series 1, the plants were grown under five K levels, employing 5, 19.5, 39, 97.5, and 195 p.p.m. K, as was described with other information concerning this series (**28**).

In series 2, the plants were grown from seed that had been planted in washed and sterilized sand on August 27. On September 29 the plants were transplanted into acid- and base-washed sand. Different B and K levels were applied in a 3×3 factorial design, as indicated in table I,

TABLE I

COMPOSITION OF NUTRIENT SOLUTIONS EMPLOYED IN GROWING RANGER ALFALFA UNDER DIFFERENT K AND B LEVELS*
SERIES 2

K AND B LEVELS		KNO_3	$Ca(NO_3)_2$	pH VALUES	
K P.P.M.	B P.P.M.	MOL./L.	MOL./L.	NUTRIENT SOLUTION	FINAL DRIP
10	.001	.00025	.0074	4.4	6.0
10	.5	.00025	.0074	4.4	5.9
10	5.0	.00025	.0074	4.3	6.0
195	.001	.005	.005	4.4	6.0
195	.5	.005	.005	4.4	6.1
195	5.0	.005	.005	4.3	5.9
468	.001	.012	.0015	4.4	6.1
468	.5	.012	.0015	4.4	6.1
468	5.0	.012	.0015	4.4	6.0

* Other materials supplied: Fe^{++}, 1 p.p.m.; Mn^{++}, 0.25 p.p.m.; Zn^{++}, 0.25 p.p.m.; $NH_4H_2PO_4$, 0.0005 mole per liter, and $MgSO_4$, 0.002 mole per liter. Ca was also varied in this experiment to compensate for the changes in K to maintain a constant osmotic pressure.

and set up in duplicate. All plants received 0.05 p.p.m. B and 39 p.p.m. K, until special treatments were started October 24. The plants were harvested when all but those deficient in B were in the late bud stage. Tests of the nutrient solutions and of the final drip (table I) show little variation in pH values within the series. Daily variations were less than the differences between the initial solutions and the final drip.

Fresh plant samples were frozen for expressed-sap analysis. The frozen samples were expressed for two minutes at 3750 pounds per square inch, in a Carver press. Since it was desired to determine the minerals present in solution rather than those actually soluble, no washings were made of the residue.

Chemical analyses for Ca, K, Mg, P, S, Mn, and Fe were made on leaves, stems, roots, and sap, according to the procedures outlined by TOTH *et al.* (**26**). B and N determinations were made according to A.O.A.C. methods. All leaf analyses included the petioles.

The major elements were calculated to m.e. cations or anions per 100

gms. dry matter. Minor elements such as Fe, Zn, Cu, and B were disregarded in the cation and anion calculations because their equivalent values are not large enough to significantly influence the results. All N was assumed as the anion, NO_3^-; and P and S were considered as $H_2PO_4^-$, and SO_4^{--}.

Series 1 involved a study of K, and series 2 of both K and B. Ca was varied in both experiments to compensate for changes in K. The plants in series 1 were grown in the spring and harvested on April 29, whereas those in series 2 were grown in the fall and harvested on December 17. All the N (196 p.p.m.) of series 1 was supplied as the nitrate, whereas in series 2 seven of the 217 p.p.m. N was supplied as $NH_4H_2PO_4$. The advantages derived from more precise pH control were believed important enough to include this small quantity of NH_4. It was recognized that the NH_4 would influence the final result, but its effects were minimized by maintaining the pH values in the acid range. The plants of series 1 were supplied 31 p.p.m. P and those of series 2 only 15.5 p.p.m. The highest

TABLE II

TOTAL CATION, ANION, B, FE, AND MN CONCENTRATIONS IN RANGER ALFALFA AND CATION-ANION RATIOS OF TOP PORTIONS

SERIES 1

K LEVEL	TOTAL* CATION	NO_3^{-}**	$H_2PO_4^{-}$**	SO_4^{--}**	TOTAL ANION	CATION / ANION	B	FE	MN
p.p.m.	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*Ratio*	*p.p.m.*	*p.p.m.*	*p.p.m.*
				TOPS					
5	165	261	25	23	309	.54	28	104	73
19.5	166	260	22	20	302	.55	32	105	61
39	152	257	19	18	294	.52	34	109	70
97.5	156	270	18	18	306	.51	40	98	64
195	163	273	16	18	307	.53	35	92	68
				LEAVES (INCLUDING PETIOLES)					
5	211	335	29	30	394	.54	40	144	114
19.5	212	352	24	26	402	.53	47	139	100
39	187	360	19	26	405	.46	53	150	122
97.5	190	370	18	24	412	.46	67	138	110
195	187	372	13	25	410	.46	53	130	110
				STEMS					
5	87	139	19	13	171	.51	8	40	4
19.5	110	143	19	12	174	.64	14	65	13
39	113	154	19	10	183	.62	14	65	12
97.5	117	162	18	9	189	.62	11	53	8
195	135	162	19	10	191	.71	14	48	18

* Individual cation contents reported previously (28).

** All N, P, S, present in the tissues calculated as m.e. in terms of the ions in the nutrient solution. All cation and anion values reported as m.e. per 100 grams dry matter. To translate m.e. per 100 gm. to percentages, multiply the m.e. value of K by 0.039, Ca by 0.020, Mg by 0.012, N by 0.014, P by 0.031, and S by 0.016. To translate percentages into m.e. per 100 gm. dry matter, divide percentage by the m.e. weight. Thus 1 per cent. K becomes 25.6 m.e. per 100 gm. and 1 m.e. K per 100 gm. becomes 0.039 per cent.

K level was lower in series 1 than in series 2 and, consequently, the highest Ca level was higher. The osmotic pressures of all solutions were constant for each series, although those in series 1 were about 2 per cent. lower than those of series 2.

Results

Certain of the data from series 1 have already been reported (**28**). Additional chemical analyses of these plants are given in table II of the present paper.

In series 2, no B-deficiency symptoms had developed by November 15. This element was eliminated, therefore, from all 0.001 p.p.m.-B treatments. B-deficiency symptoms appeared in eight days. They were more severe as the K concentration of the solution increased. These results correspond with those obtained by Shive and his students (**24**). Decreased height was the only visible expression of K-deficiency, in contrast to the typical spotting observed in series 1. B-toxicity symptoms appeared only in the 10 p.p.m. – K + 5 p.p.m.-B series (low-K and high-B). At harvest time, buds were present on all plants, except those of the low-B treatments. Buds that had formed on some low-B plants abcissed.

B-deficiency in plants receiving low- and intermediate-K solutions was characterized by hardening, yellow-mottling, and crinkling of the terminal leaves. Plants receiving high-K solutions showed more severe B-deficiency and their terminal leaves had a large amount of red pigmentation. The B contents of the leaves of plants growing in the low-, medium-, and high-K solutions were 26, 19, and 10 p.p.m., respectively. The reddening when K concentration was highest may have been a result of more severe deficiency. Complete data on series 2 are recorded in tables III, IV, V, and VI.

TABLE III

DRY-WEIGHT YIELDS OF TOPS AND ROOTS*, LEAF PERCENTAGES, AND COLOR OF ROOTS OF RANGER ALFALFA PLANTS

SERIES 2

K AND B LEVELS		TOPS	ROOTS	LEAF (PORTION OF TOP)	COLOR OF ROOTS
K	B				
p.p.m.	*p.p.m.*	*gm.*	*gm.*	%	
10	.001	4.3	1.5	51.0	Brown
10	.5	4.6	1.1	48.5	Brown
10	5.0	4.8	1.5	47.5	Brown
195	.001	4.1	1.8	53.0	White
195	.5	7.9	1.2	48.5	White
195	5.0	6.8	1.0	43.5	White
468	.001	3.1	1.5	53.5	Brown
468	.5	7.1	1.2	44.0	Brown
468	5.0	7.0	0.9	47.5	Brown

* In grams per plant.

TABLE IV

INDIVIDUAL AND TOTAL CATION- AND ANION-EQUIVALENT CONCENTRATIONS* AND RATIOS OF RANGER ALFALFA PLANTS; AND B, FE, AND MN CONCENTRATIONS OF LEAVES, STEMS, ROOTS, AND TOPS

SERIES 2

K AND B LEVELS		K	CA	MG	TOTAL CATION	NO_3^-	$H_2PO_4^-$	SO_4^{--}	TOTAL ANION	CATION / ANION	B	FE	MN
K	B												
p.p.m.	*p.p.m.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*Ratio*	*p.p.m.*	*p.p.m.*	*p.p.m.*
						TOPS							
10	.001	24	101	43	168	291	20	18	329	.51	17	146	54
10	.5	23	98	36	157	287	20	19	326	.49	93	95	60
10	5.0	23	95	36	154	283	19	17	319	.48	197	98	55
195	.001	94	65	18	177	323	18	15	356	.50	14	142	59
195	.5	88	57	15	160	293	17	17	327	.49	91	105	45
195	5.0	94	48	12	154	279	15	16	310	.50	127	115	39
468	.001	124	34	23	181	293	19	21	333	.54	10	137	22
468	.5	118	28	18	164	277	13	15	305	.54	59	88	30
468	5.0	127	30	21	178	309	15	15	339	.53	110	103	36
						LEAVES							
10	.001	29	154	34	217	389	17	26	432	.50	26	215	93
10	.5	27	159	34	220	410	21	32	463	.48	155	155	107
10	5.0	26	159	33	218	417	21	28	466	.47	360	155	107
195	.001	85	93	19	197	380	17	21	418	.48	19	205	98
195	.5	81	88	21	190	412	18	26	456	.42	150	170	78
195	5.0	83	79	21	183	422	17	29	468	.40	240	200	78
468	.001	110	50	30	190	350	17	29	396	.48	10	188	38
468	.5	113	45	30	188	415	15	27	457	.41	110	145	58
468	5.0	113	45	32	190	415	16	24	455	.41	200	163	63

TABLE IV—*Continued*

K AND B LEVELS		K	Ca	Mg	TOTAL CATION	NO_3^-	$H_2PO_4^-$	SO_4^{--}	TOTAL ANION	CATION / ANION	B	Fe	Mn
K	B												
p.p.m.	*p.p.m.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*Ratio*	*p.p.m.*	*p.p.m.*	*p.p.m.*
						STEMS							
10	.001	18	44	53	115	195	23	9	227	.51	7	73	13
10	.5	19	39	38	96	170	20	7	197	.49	34	38	13
10	5.0	16	37	38	91	162	17	8	187	.49	50	46	8
195	.001	103	33	16	152	236	20	8	264	.58	9	73	15
195	.5	95	27	10	132	183	15	8	206	.64	35	42	13
195	5.0	102	24	7	133	170	14	6	190	.70	40	50	8
468	.001	141	15	15	171	226	21	12	259	.69	11	80	5
468	.5	121	14	9	144	169	12	6	187	.77	19	42	8
468	5.0	139	17	10	166	214	15	7	236	.70	26	49	13
						ROOTS							
10	.001	6	9	22	37	185	10	14	209	.18	4	405	12
10	.5	4	12	23	39	186	11	9	206	.19	14	388	13
10	5.0	4	12	20	36	183	9	12	204	.18	12	438	18
195	.001	25	7	11	43	220	11	11	242	.16	10	280	15
195	.5	35	9	10	54	206	11	13	230	.24	24	271	22
195	5.0	42	11	10	63	195	12	12	219	.29	28	330	22
468	.001	38	3	13	54	224	12	14	250	.22	8	300	17
468	.5	40	5	12	57	183	10	12	205	.28	22	348	15
468	5.0	44	6	12	62	201	11	13	225	.28	24	378	19

* See notes, table II.

TABLE V

CATION AND ANION CONCENTRATIONS AND RATIOS, AND B IN ALFALFA PLANTS (INCLUDING ROOTS); AND CATION RATIOS OF TOP PORTIONS

SERIES 2

K AND B LEVELS		TOP PORTION OF PLANT	TOPS + ROOTS				TOPS		
K	B		TOTAL CATIONS***	TOTAL ANIONS***	CATION / ANION	B	Ca + K / Mg	Ca + Mg / K	K + Mg / Ca
p.p.m.	*p.p.m.*	%	*m.e.*	*m.e.*	*Ratio*	*p.p.m.*	*Ratio*	*Ratio*	*Ratio*
10	.001	74.2	134	298	0.45	14	2.88	6.00	0.66
10	.5	80.6	134	301	0.45	78	3.36	5.84	0.60
10	5.0	76.3	127	291	0.44	154	3.30*	5.72*	0.63*
195	.001	69.6	136	320	0.43	12	8.84	0.89	1.73
195	.5	87.0	146	314	0.47	82	10.31	0.81	1.78
195	5.0	87.4	142	297	0.48	114	11.80	0.64	2.20
468	.001	67.5	140	306	0.46	9	6.88**	0.46**	4.30**
468	.5	85.5	148	292	0.50	54	8.10	0.39	4.86
468	5.0	87.0	162	325	0.50	97	7.15	0.41	4.87

* B-toxicity.
** Extreme B-deficiency.
*** Cations are the mineral cations, K, Ca, Mg only. All N was calculated as the anion. Per 100 grams dry tissue.

TABLE VI

pH VALUES, P, B, Fe, AND CATION CONCENTRATIONS IN EXPRESSED SAP; AND CATION SUMMATION VALUES IN SAP AND RESIDUES* OF TOPS OF RANGER ALFALFA

SERIES 1

K AND B LEVELS		pH	P	B	Fe	K	Ca	Mg	CATIONS IN SAP	CATIONS IN RESIDUE
K	B									
p.p.m.	*p.p.m.*		*m.e.*	*p.p.m.*	*p.p.m.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*
5	.5	5.52	9	20	1.0	11	64	21	96	69
19.5	.5	5.65	8	14	1.4	25	50	18	93	73
39	.5	5.85	7	13	2.8	38	36	16	90	62
97.5	.5	5.95	5	16	5.2	49	34	12	95	61
195	.5	6.00	4	7	11.0	55	25	9	89	74
					SERIES 2					
10	.001	5.85	8	5	0.5	16	53	21	90	78
10	.5	5.87	6	51	0.7	15	58	16	89	68
10	5.0	5.98	5	134	0.2	13	51	14	78	76
195	.001	5.97	6	8	2.0	60	20	11	91	86
195	.5	5.99	5	35	3.4	64	26	8	98	62
195	5.0	6.01	5	90	1.9	68	19	7	94	60
468	.001	5.90	9	8	2.0	78	16	13	107	74
468	.5	5.98	7	53	4.0	79	13	10	102	62
468	5.0	5.98	6	85	1.0	85	14	10	109	69

* Cations and P are reported as m.e. per 100 gm. dry matter. All P in sap calculated as $H_2PO_4^-$. All values for sap are on the basis of the original oven-dried plant material.

Yields of the plants grown in series 1 increased with increasing K, including the highest K concentration. These highest-K levels cannot be regarded as involving luxury consumption, even though the K content of the plants was about 3 per cent. Maximum yield of dry matter of the tops and normal color of the roots were not obtained until the K content of the plant tops had exceeded 2 per cent.

Lower yields were obtained at all the low-B and low-K levels in series 2 (table III). When the K content of these plants exceeded 3 per cent., yields were not increased, regardless of the B content of the plants.

As reported earlier (**28**), increasing the K concentration sometimes resulted in increased total cation-equivalent values of alfalfa. When K was increased in the nutrient solution, larger proportions of the total K were found in the stems. This phenomenon was pronounced in series 2 when luxury consumption of K occurred. Total cation-equivalent values in the latter stems ranged between 91 and 171 m.e. per 100 gms. dry tissue, these values being within the lowest- and highest-K levels, respectively. This trend was independent of the B concentration of the nutrient medium. When the cation-equivalent values were calculated to a whole-top basis, these differences tended to disappear, because the tops of the low-K plants were smaller and had higher leaf percentages. The correlation coefficient between dry weight and leaf percentages for these plants was –0.81, and highly significant.

Alfalfa leaves usually have higher and more constant cation-equivalent values than the stems. Smaller plants have higher leaf percentages than larger plants. To maintain cation-equivalent constancy on the whole-plant basis, therefore, the cation-equivalent value of the stems must be flexible. This occurred with different K levels, and is one type of counterbalancing factor.

The data on series 2 indicate that the cation-equivalent values in the leaves of plants in low-K solutions were slightly higher. Certain of the cation-equivalent values were also high on the whole-top basis, notably when the solutions were high in K and low in B (table IV). These two influences indicate that another counterbalancing factor is operating.

Differences in the percentage content of minerals in plants occur because of (a) differences in the total and relative quantities of the several mineral elements supplied, (b) their concentration or dilution within the plant as a result of increased or decreased carbohydrate production or of increased respiration, and (c) their differential distribution among the various organs. Differences arising purely from differential distribution are eliminated if the composition of the entire plant is considered as a unit.

Of particular interest are the cation-anion equivalent ratios (K + Ca + Mg : N + P + S) of these two series of plants. It was observed in series 1 that cation-anion ratios in the leaves tended to decrease with increas-

ing K in the nutrient solution (table II), whereas those in the stems tended to increase. The ratios for the leaves and stems combined were considerably more constant.

Referring to the reasons for differences in composition: (a) the varying K treatments did not alter the cation-anion equivalent ratios; (b) the ratios for the whole plants were not changed by carbohydrate dilution; and (c) the ratios varied oppositely within leaves and stems by reason of distribution phenomena.

No one part of the plant can be relied upon to represent the composition of the entire plant. Accordingly, in series 2, the cation-anion equivalent ratios were determined for the entire plants, including roots (table V). These ratios were more constant than those for the tops alone. This is remarkable since increased K-uptake resulted in a marked increase in total cation-equivalent values for the stems; increased B-uptake resulted in a decrease in cation-anion equivalent ratios in the leaves and a complication of the ratios in the stems; and both increased K- and increased B-uptake resulted in increased cation-anion equivalent ratios in the roots. The tendency for the cation-anion equivalent ratios of the whole plant to be constant helps to explain many of the differences observed in the chemical composition of the various plant parts.

Cation-equivalent values for the plant tops in series 2 (table IV) are not constant. When the cation content of the plant was increased, however, so also was that of the anions. Even the Fe content was increased in almost the same proportion. Cation-anion equivalent ratios of all plants, including those showing deficiency symptoms, were similar.

The differences in cation-equivalent values are undoubtedly the result of increased concentration within the plant by reason of a depression in carbohydrate accumulation. They do not, therefore, contradict cation-equivalent constancy. This same phenomenon existed between different cuttings of alfalfa (**29**). The relative concentration of carbohydrate materials in these plants is assumed from variations in the amounts of cations and anions when the cation-anion ratio for the whole plant is constant.

The slight increase in cation-equivalent values of the leaves of the low-K plants is not a contradiction of the cation-equivalent concept but is a result of adjustment to metabolic activity, to distribution of products, and to relative proportion of leaves, stems, and roots.

Constancy of cation-anion equivalent ratios in plants from a particular nutrient medium suggests that laws for absorption of ions might be established. Cations and B, but none of the anions, were varied in the nutrient medium. Final interpretation must await similar anion studies. In this respect LUNDEGARDH (**13**) has reported, from studies with single-salt solutions, that cation-anion equivalent ratios decrease as salt concentrations increase.

In series 1, the plant's concentration of P and Mg decreased as that of its K increased. Mg-P relationships have been suggested (**27**). Results of series 1 indicated, however, that the cation-P relationship could be the result of any or all of the cations, K, Mg, or Ca. No single cation value, or combination of values, divided by P was constant for all five treatments. When K concentration was increased in the plant, that of Ca and Mg decreased. When K concentrations in the nutrient solution were increased from 195 to 468 p.p.m. in series 2 and that of Ca was reduced an equal number of equivalents, the Mg as well as the K concentration in the plant increased. Thus high levels of K in the nutrient solution will not necessarily induce Mg deficiency in plants (**3, 23**) unless the level of Ca is also high or that of Mg is very low. In series 2, the evidence was at variance with the increasing-K and decreasing-P trends observed in series 1. The P concentration did not follow that of Ca in the latter plants, providing evidence that Mg concentration in plants is positively correlated with that of P. Data previously reported (**28**, table IV) showed the same trends for Mg when the nutrient solutions were high in K and low in Ca. P values for these plants, also previously reported (**17**), followed the Mg rather than the Ca or, inversely, the K values.

The S concentrations tend to follow the same trends as those of P (tables II, IV). Decreasing concentrations of these two elements with increasing K in the plants of series 1 resulted in equivalent increases in N. These increases were relatively small because N usually supplied by far the largest proportion of the anions. These adjustments resulted in almost constant anion equivalent values and cation-anion equivalent ratios both in the leaves and in the whole plant.

The data in series 2 suggest cation-B relationships. The B concentration of both the leaves and the complete tops decreased with increasing K and decreasing Ca at all B levels. At the low-B level, the plants with the highest K concentration contained the least B, and they were the most severely B-deficient. Also, at the high-B level, the low-K (10 p.p.m.) + high-Ca plants contained the most B and were the only plants showing B-toxicity. The B range in the leaves at this B level was from 360 to 200 p.p.m., and from 154 to 97 p.p.m., for the whole plant (table V). Variations in the plants' contents of Ca and K greatly influenced their B uptake.

Higher B content in plants was associated with a higher $\frac{Ca + Mg}{K}$ ratio and with B toxicity. SHEAR *et al.* (**23**) sometimes obtained the same results for tung leaves, but more commonly B toxicity resulted with high $\frac{Ca + K}{Mg}$ ratios. In alfalfa, lower B content and extreme B-deficiency were associated with very low $\frac{Ca + Mg}{K}$ ratios (table V). Shear and his collab-

orators state that high $\frac{K+Mg}{Ca}$ ratios have little influence on B toxicity. In the present work, they were associated with B deficiency.

The B for series 1 was supplied at a single satisfactory level. In contrast to series 2, the B content of the low-K plants was slightly lower than that of the high-K plants. B behaves differently under various photoperiods (**15, 21**).

B appeared to have little influence on the contents of individual cations in the tops. At the higher-K levels, increasing B resulted in slightly larger contents of K and Ca in the roots. This factor may be of practical importance in the longevity of this plant.

The Mn content of the plants in the highest-K solutions was reduced in the tops and increased in the roots (table IV). Such differences in distribution of nutrient elements might easily be mistaken for differences in absorption.

Constancy in cation-equivalent values of sap has been suggested (**28**). Interpretations of expressed sap analyses are fraught with difficulties inherent in this type of work. In the high-K plants of series 2, cation-equivalent values of the sap were increased slightly by reason of the higher K. In both series, cation-equivalent values were equally constant in the residue and sap (table VI).

When B was varied at constant cation levels in the nutrient solution, no significant differences were noted in the cation content of the sap of the plant. The B content of the sap tended to be a function of the total B. A larger percentage of the total B was in the expressed sap of the plants of series 2 (April) then in those of series 1 (December).

The Fe content of the sap in the plants grown in the low-K solutions was much smaller than in that of the plants in the higher-K solutions (table VI). The first abnormal response that was observed for plants of the low-K solutions in the first series was a typical Fe deficiency, which was overcome by doubling the Fe content of the nutrient medium. Ca, K, and P relationships have been correlated with Fe deficiency (**31**). HOFFER and TROST (**8**) show that Fe accumulates in the nodes of corn plants when K is deficient. This may explain the increased Fe content of the roots of the plants of the low-K solutions. SHEAR and CRANE (**22**) state that excess accumulations of any of the cations can induce Fe deficiency. High concentrations of B further reduced the quantity of Fe in the sap.

Increasing K in the nutrient solution tended to result in lower P, both in the tops and in the sap. Excess P is believed (**31**) to precipitate Fe within the plant. The observed influence of K on Fe could have been the result of the increased P in the plants. The pH of the sap was lower in series 1 when the K was low. This was true generally in series 2, but the differences were inconsistent and of doubtful significance. The P in the sap may control its pH value, and pH-P-Fe effects could result in the iso-

electric precipitation of Fe. In contrast to these data, pH values of plant sap obtained by LOEHWING (**11**) increased with lower plant concentrations of K.

Discussion

Cation-anion equivalent ratios in plants grown in sand-culture experiments using inorganic-nitrogen sources were practically identical with those reported (**29**) for field-grown alfalfa where the plants obtained N from symbiosis with Rhizobium. Even though legumes obtain atmospheric N by the action of nodule bacteria, the mechanism of cation absorption may be similar in both cases. This might suggest a separate mechanism for cation and anion absorption, which has been proposed by LUNDEGARDH (**14**).

Knowledge of cation-anion ratios could be highly useful in helping solve certain problems of soil fertility and plant nutrition. Such problems as the mechanism of ion absorption by roots, genetic differences in relation to mineral content; influence of unessential ions, such as Na and Cl; changes in composition with age; effect of osmotic pressure and pH of the nutrient medium on growth and composition; and differences in the nutritive effect of NH_4^+ and NO_3^- might be further elucidated.

Since SHIVE *et al.* (**24**) had shown that B functions in keeping Ca in a soluble form in the plant; since K is almost entirely in the soluble form in plants; and since the cations are believed to have, in part, a common function in the plant (**25**); it was thought that an increase of B in the nutrient solution, at moderately low K levels, might lessen the K requirement by keeping Ca in solution and thus releasing K for other functions. This did not prove to be so. B had little influence on the amount of Ca found in the sap, and increasing B in the nutrient solution to the toxic level did not increase yields when the K in the plants was near the 1 per cent. critical level set by HUNTER *et al.* (**9**). It should be noted that there is a difference between soluble Ca and that present in the sap, although the latter unavoidably includes some of the former.

Dry-matter production of alfalfa in these experiments was increased up to 3 per cent. K in the plant tops, and root growth was not ideal below 2-per cent. K. This suggests that the 1 per cent. critical K level set for field alfalfa was the point where other limiting factors became operative. JACKSON *et al.* (**10**) found that alfalfa had poor competing power with other plants when its K content was below 3 per cent. It may not be practicable to maintain the K at this level, a value midway between 1 and 3 per cent. being more logical for field production of this crop. Plants having very high K concentrations are low in Ca. This could impair their nutritional value as forage.

One plant part or organ cannot be used as an index for the composition of the entire plant. Differences in one organ are often related to

differences in distribution within the plant. Many investigators select morphologically similar leaves for tissue-testing procedures (**12, 23, 25**). Shear *et al.* defend this procedure on the basis that leaves are the plant's manufacturing organs, that all the elements influencing the leaf processes must be present in proper balance, and that, from a knowledge of the magnitude of all the elements in the leaves, nutritional unbalance can be detected. A flaw in this reasoning is that mobile elements move to the growing regions and thereby deplete other plant parts. This is especially noticeable with K, which is believed to have great importance in the roots of alfalfa in initiating new growth after each cutting, and as an aid to survival during the winter (**16**). This objection can be overcome in many cases by selecting proper morphological parts for study. The data for the K content of alfalfa given in table IV of reference (**28**) is a striking example of the distribution of this element within this plant. In the first treatment, the K was near the 1 per cent. critical level. If the terminal leaves or terminal stems alone had been analyzed, the results would have shown that the plants were abundantly supplied with K, whereas the plant as a whole was near the critical level. If the meristematic regions of the alfalfa plant are provided with K to the 2 per cent. level by depleting it in the rest of the plant, this may be a more nearly optimum level than 1 per cent. In the other treatments of that experiment, K was distributed more uniformly throughout the plant.

The nature of the cation-anion ratio suggests that nonessential elements may exert important influences on plant growth. This has been discussed by various workers (**20, 23, 29**) but requires further study.

Reference was made to contradictions in K-B and Ca-B relationships. A plant maintaining cation-equivalent constancy may behave very differently from one not maintaining such constancy when one cation is varied at different levels of B nutrition. There was indication that B was similar to P in an Mg-P trend. Extremes of both Ca and K greatly influence the Mg content of alfalfa.

Most studies of Ca versus B have neglected the fact that other elements in the plant vary when Ca varied in the nutrient medium. Variations in K and Mg in the nutrient medium undoubtedly have an important bearing on the final result. Results will be contradictory if one experiment is designed in which all the cations vary greatly in the plant, whereas in another they vary little. Recent work (**4**) on Ca-B relationships involved an increase of Ca in the nutrient solution by adding increments of $CaCl_2$ and $CaSO_4$. This effected more than a twofold difference in the osmotic concentration from the lowest to the highest Ca concentration. The unknown influence of this and of the simultaneous increase in Cl in the nutrient medium and the unmeasured changes in K and Mg in plant tissue all contribute to the difficulty in interpreting results of this type. Where large amounts of K

have been supplied, it is often difficult to induce large changes in the Ca content of plants, even when large amounts of Ca are supplied.

ALBRECHT (**1**) and RICHARDS (**20**) have suggested that low-K plants are "proteinaceous" whereas those high in K are "carbonaceous." Plants grown at low nutritional levels in this work were smaller and had higher leaf percentages. Superficially, this might suggest a proteinaceous plant. When the whole plant was considered, however, cation-anion ratios were similar in all plants. The N and other elements in some of these plants were "concentrated" as a result of decreased carbohydrate accumulation. The proteinaceous nature of plants grown in low-K and high-Ca solutions cannot be considered as a fundamental change in their nature.

At similar B and K levels in the nutrient solution, more B was absorbed in December than in April, confirming the results of Eaton for alfalfa (**6**). For a period of about a week in early December, the greenhouse temperature was somewhat lower than desired, and, in this condition, the B-deficient plants in series 2 resumed growth. This continued until the temperature was corrected. The B requirement apparently depends upon temperature as well as photoperiod.

Summary

Ranger alfalfa was grown in sand culture at different K levels and at K and B levels that varied simultaneously. The following results were obtained:

Yields were increased with increasing K up to a K content of 3 per cent. in the plant, which is considerably higher than the critical level set for field-grown alfalfa.

B-deficiency was characterized by hardening, crinkling, and yellow mottling of terminal leaves in plants grown in low-K solutions. Plants grown in high-K solutions showed more severe deficiency, and their terminal leaves had red pigmentation from the tips inward. The B content of the leaves of deficient plants was 26 p.p.m. for the plants in the low-K solutions and 10 p.p.m. for those in the high-K solutions.

B toxicity was evident only on plants grown in the 5 p.p.m.–B + 10 p.p.m.–K solutions. This was characterized by a brown, necrotic fringe on the leaves of the lower part of the plant. The leaves of the B-injured plants contained 360 p.p.m. B, compared with 240 and 200 p.p.m. for plants in the higher-K solutions at the same B level. Plants in the high-K solutions contained less B and were more severely B-deficient when B was lacking, whereas those in the low-K solutions accumulated more B and showed more injury when B was abundant.

The differences observed in plant composition when the mineral nutrition was varied is explained on the basis of three points, *i.e.*, (a) differences in the concentrations and ratios of minerals supplied to the plant,

(b) relative concentration or dilution within the plant from differences in carbohydrate accumulation, and (c) differential distribution among the various plant parts. These factors complicate diagnosis by chemical analysis of foliar parts.

Cation- and anion-equivalent constancies were functions of carbohydrate accumulation, leaf, stem, and root percentages, and the distribution of the ions throughout the leaf, stem, and root. These counterbalanced each other.

Cation-anion equivalent ratios for entire plants, including roots, were remarkably constant, despite variations in K and B, size of tops and roots, and leaf percentages. This was not true for any plant part alone.

The concept that luxury consumption of K results in a proportionately larger concentration of K and a higher cation-equivalent value in stems was further substantiated.

When the amounts of K and Ca only were varied in the nutrient solution, the K, Ca, Mg, N, P, S, and B contents of the plant varied, and the contents of all these, plus those of Fe and Mn, in the expressed sap also varied.

At constant, optimum B levels, increase of P and S in low-K plants resulted in equivalent decreases in N. As a result, constancy in anion-equivalent values and in cation-anion equivalent ratios was maintained.

At all B levels, plants contained higher Mg concentrations when grown in nutrient solutions containing 468, 60, and 48 p.p.m. K, Ca, and Mg, respectively, than when grown in those containing 195, 200, and 48 p.p.m. of these elements. This was despite large increases in the K content of the tissues. The P concentration was more closely related to that of Mg than to that of the other cations.

Plants that were low in K contained much less Fe and more P in the sap than those that were high in K. The Fe content of the sap was further reduced in the high-B plants.

Plants that were low in K were not necessarily "proteinaceous." Nutritionally-deficient plants had high leaf percentages and some were high in N and mineral elements, but this was a result of low carbohydrate accumulation.

Possible value of the use of cation-anion ratios in the solution of problems of plant nutrition and soil fertility are discussed.

The authors wish to express appreciation to members of the Plant Physiology Department of the New Jersey Agricultural Experiment Station for assistance and for use of materials in conducting this study.

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CARBOHYDRATE FRACTIONS OF LEMON PEEL[1]

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Received January 10, 1949.

Introduction

The peel of citrus fruits is physiologically important in fruit growth and maturity in that the vascular system is confined chiefly to the mesocarp (albedo), which thus serves the important function of transporting water and solutes from the trees to the juice vesicles of the pulp. To understand the principles of the biochemical mechanisms occurring in the fruit during growth and development, one has to know the chemical composition of the peel and the relation between the constituents. Such data are also essential for the understanding of certain physiological relations between peel and pulp, and for the understanding of the metabolic reactions of the peel under storage conditions. The phase of the work which has to do with the organic acids and buffer properties of citrus peels has already been published (**21**).

The present studies have been concerned with the carbohydrate fractions of lemon fruit peel. Samples of peel from lemons have been thoroughly extracted with 80 per cent. ethyl alcohol and separated into alcohol-soluble and alcohol-insoluble fractions. The alcohol-soluble fraction contains chiefly the mono- and di-saccharides; it also contains other substances such as essential oils and waxes, and various concentrations of undetermined substances. The alcohol-insoluble fraction contains the cell-wall constituents, such as cellulose, lignin, pectin, and hemicellulose (sometimes designated as polysaccharides or acid-hydrolyzable material). These substances, particularly pectin and hemicellulose, appear to be closely related. The pectins are not usually considered as part of the carbohydrate reserve of the plant. The readily available carbohydrate fractions serving as sources of energy consist of the total-soluble-sugars and the starch-dextrin fractions. The carbohydrate material of the cell walls is not necessarily composed of condensation products of the simple sugars, but may consist of uronic acids united with the hexose and pentose sugars. Pectins belong to this general class of compounds, and any hemicellulose fraction that may accompany the pectins on weak-acid extraction of the tissues probably contains a uronic acid group. Cellulose, hemicellulose, and lignin compose the structural, and not the energy-yielding, units of the tissues; consequently, they are relatively inert to the metabolic activity of the cells.

Materials and methods

Alcohol-insoluble solids and alcohol-soluble solids were determined on

[1] Paper no. 601, University of California Citrus Experiment Station, Riverside, California.

the peel from 50 to 100 lemon fruits having diameters of approximately 2½ inches. For moisture determinations, disks of peel were removed from the equatorial region of the fruit with a cork borer (no. 9). Other disks were taken from the peel, weighed, and used for the quantitative determination of the alcohol-insoluble and alcohol-soluble solids. A much larger sample of the remaining peel was used for obtaining a large experimental sample of alcohol-insoluble material.

For quantitative determinations on the small disk samples, the peel was ground in a Waring blendor in the presence of 95 per cent. alcohol, heated to boiling and allowed to stand overnight in 300 ml. of 80 per cent. alcohol, a correction being made for the moisture in the sample. Two additional extractions were made with hot 80 per cent. ethyl alcohol, and for each extraction the samples remained in the extractant for 24 hours. The insoluble material was filtered and washed, first with 95 per cent. ethyl alcohol and finally with petroleum ether. The colorless product was dried to constant weight at 65° C and weighed quantitatively. The percentages of alcohol-insoluble solids were calculated from this weight.

Pectin was extracted from the alcohol-insoluble solids by placing 0.50 gm. of the material in a 150-ml. beaker and adding 50 ml. of hot water. The mixture was gently heated to slow boiling for a 10-minute extraction period. While hot, the extract was filtered with suction on a hardened filter paper (11-cm., Whatman no. 50). The solid material was returned to the beaker with 50 ml. of boiling water, and the process was repeated. A third extraction was made in the same way, all of the filtrates being received in the same flask. The combined solution was filtered by suction through a small, soft paper (Whatman no. 42), and the filter washed with hot water. The extract was diluted to 300-ml. volume.

Pectin in the water extract was determined as calcium pectate by the method of CARRÉ and HAYNES (5), with slight modifications. The pectin was saponified by adding 6 ml. 5 N NaOH to the 300-ml. aqueous extract, and the solution was allowed to stand for one hour. Fifty milliliters N acetic acid was added, with stirring, and, after five minutes, 50 ml. M calcium chloride was added. The solution was allowed to stand one hour, or longer, for the complete precipitation of calcium pectate. The precipitate was filtered by suction through a hardened paper (11-cm., Whatman no. 50) on a Büchner funnel. When the volume in the funnel had been reduced to about 50 to 75 ml., the precipitate was washed with boiling water until the filtrate was free of chlorides. (The volume of the material in the funnel must not be less than 50 ml. at any time; otherwise, filtering becomes too slow and washing difficult.) When washing was complete, the precipitate was sucked dry enough so that it would adhere to the paper when lifted from the funnel. The matched paper was then wet with distilled water, and both papers were placed in an oven and dried to constant weight at 105° C, using the balanced paper as tare. The dried calcium pectate loosens from the paper and is readily removed as a thin film for analysis.

The material remaining after the three extractions with boiling water was then extracted in a similar manner with three successive 50-ml. portions of hot $\frac{N}{75}$ HCl at pH 2.5. The combined solutions were filtered through soft paper and diluted to 300 ml. Sufficient concentrated HCl was added to make a 10 per cent. solution. This precipitated pectinic acid in a form which could be readily filtered, with suction, through a hardened paper. Without washing, the paper and precipitate were returned to the beaker. About 100 ml. of hot water was added, together with one drop of phenolphthalein solution and sufficient NH_4OH to produce a faint pink. The pectinic acid readily dissolved. The paper was returned to the funnel, and the solution drawn through it two or three times to dissolve any precipitate adhering to the funnel. Finally, it was washed with hot water. The total volume was again about 300 ml. Calcium pectate was precipitated and treated as in the water extract, except that it was filtered on a 7-cm. paper.

The material left after the dilute HCl extractions was similarly extracted three times with 50 ml. of 1 per cent. ammonium citrate. The combined extracts were filtered and diluted to approximately 150-ml. volume. The extract was made neutral to phenolphthalein, and the calcium pectate determined as already described.

The residue remaining after the ammonium citrate extraction was washed with hot water and transferred to a weighed watch glass and dried at 105° C. This residue was designated as the cellulose and hemicellulose fraction.

Alcoholic extracts and washings were combined and freed of alcohol by evaporation on a steam bath. The residue was extracted with warm water to dissolve the soluble sugars. The aqueous extract was diluted to a definite volume, and aliquot portions were taken for analysis.

Sugar determinations were made by the Hagedorn and Jensen method (**8**, **9**) as modified by BLISH (**2**, **3**). The strength of the reagents employed by Blish was satisfactory for determining the reducing and total sugars as glucose when the values ranged from 3 to 10 mg. in 10 ml. of peel extract. When necessary, the samples were diluted so that the values fell within this range. This rapid method was used because comparative tests showed that it gave comparable results for the quantity of sugar in the sample. The glucose factor of the reagents was determined with a sample furnished by the National Bureau of Standards.

Uronic acids of the samples were estimated by determining carbon dioxide by the Lefevre and Tollens method (**13**) as modified by DICKSON, OTTERSON, and LINK (**6**).

Methoxyl groups were determined by the saponification procedure of ROMEO (**19**). Values obtained by this method compare favorably with those obtained by the Zeisel method (**23**, **24**).

Furfural determinations were made by the colorimetric procedure described by BRYANT, PALMER, and JOSEPH (**4**).

TABLE I

ANALYSIS OF ALCOHOL-INSOLUBLE AND ALCOHOL-SOLUBLE SOLIDS OF WHOLE LEMON PEEL

Sample	Moisture (fresh wt.)	Alcohol-insoluble solids				Alcohol-soluble solids			Water-soluble sugars (as glucose) in alcoholic extract			
		Total (dry wt.)	CO_2 produced on hydrolysis with 12 per cent. HCl		Methoxyl content	Total (dry wt.)	CO_2 produced on hydrolysis with 12 per cent. HCl		Reducing (dry wt.)	Sucrose (dry wt.)	Total	
			Dry wt.	A.I.S.* basis			Dry wt.	A.S.S.* basis			Dry wt.	A.S.S.* basis
	%	%	%	%	%	%	%	%	%	%	%	%
Fresh green lemons from experimental plots												
A	81.63	56.78	4.63	8.16	5.70	43.22	0.273	0.632	21.59	4.91	26.50	61.31
B	81.64	55.53	4.71	8.48	5.48	44.47	0.227	0.510	19.85	5.75	25.60	57.57
C	81.44	56.20	4.85	8.63	5.62	43.80	0.245	0.559	21.60	4.76	26.36	60.18
D	81.50	56.22	4.83	8.59	5.55	43.78	0.235	0.536	21.60	5.58	27.18	62.08
Mean	81.55	56.18	4.75	8.47	5.59	43.82	0.245	0.559	21.16	5.25	26.41	60.29
Mature lemons from packing-house storage												
A	78.68	61.65	4.96	8.04	5.00	38.35	0.130	0.339	20.48	1.60	22.08	57.57
B	77.90	60.00	4.70	7.83	5.07	40.00	0.192	0.480	20.19	1.61	21.80	54.50
C	77.74	60.35	4.80	7.96	5.07	39.65	0.167	0.421	19.80	1.35	21.15	53.34
D	77.22	59.50	4.78	8.04	5.07	40.50	0.170	0.420	19.32	1.82	21.14	52.20
E	76.74	58.48	4.49	7.68	5.22	41.52	0.244	0.588	20.20	1.57	21.77	52.39
Mean	77.66	60.00	4.75	7.91	5.09	40.00	0.181	0.450	20.00	1.59	21.59	54.00

* A.I.S.—Alcohol Insoluble Solids; A.S.S.—Alcohol Soluble Solids.

TABLE II

ANALYSIS OF ALCOHOL-INSOLUBLE AND ALCOHOL-SOLUBLE SOLIDS OF LEMON PEEL ALBEDO

Sample	Moisture (fresh wt.)	Alcohol-insoluble solids				Alcohol-soluble solids			Water-soluble sugars (as glucose) in alcoholic extract			
		Total (dry wt.)	CO_2 produced on hydrolysis with 12 per cent. HCl		Methoxyl content	Total (dry wt.)	CO_2 produced on hydrolysis with 12 per cent. HCl		Reducing (dry wt.)	Sucrose (dry wt.)	Total	
			Dry wt.	A.I.S. basis			Dry wt.	A.S.S. basis			Dry wt.	A.S.S. basis
	%	%	%	%	%	%	%	%	%	%	%	%
Immature (green) lemons, 2¼ inches in diameter												
1	81.29	53.70	4.30	8.01	5.05	46.30	0.17	0.36	33.01	3.20	36.21	78.20
2	81.35	53.37	4.33	8.12	5.29	46.63	0.15	0.32	33.85	1.98	35.83	76.84
3	81.61	51.92	4.16	8.01	5.27	48.08	0.19	0.40	32.37	4.75	37.12	77.20
4	81.80	52.20	4.24	8.12	5.02	47.80	0.18	0.38	32.71	3.37	36.08	75.48
5	81.45	52.40	4.23	8.07	5.08	47.60	0.17	0.37	33.10	2.73	35.83	75.27
Mean	81.50	52.72	4.25	8.07	5.14	47.28	0.17	0.37	33.01	3.21	36.21	76.60
Mature, tree-ripened lemons, 2¼ inches in diameter												
1	81.18	60.75	5.42	8.66	5.82	39.25	0.19	0.50	29.55	7.35	36.90	94.01
2	81.17	63.80	5.80	8.82	5.87	36.20	0.19	0.54	29.70	5.04	34.75	96.00
3	80.67	62.40	5.33	8.28	5.97	37.60	0.19	0.51	29.74	6.94	36.68	97.55
4	81.04	63.60	5.50	8.39	5.87	36.40	0.19	0.55	30.20	5.86	36.06	99.07
5	80.45	63.15	5.74	8.82	5.87	36.85	0.19	0.55	31.79	4.10	35.89	97.39
Mean	80.90	62.74	5.56	8.59	5.88	37.26	0.19	0.53	30.20	5.86	36.06	96.80

Calcium was determined volumetrically by treating the oxalate with dilute H_2SO_4 and subsequently titrating the liberated oxalic acid with standard potassium permanganate.

Results

Alcohol-soluble solids of whole peel and albedo

By the procedure already described, the peel was extracted with 80 per cent. ethyl alcohol and separated into alcohol-soluble and alcohol-insoluble fractions. This concentration of alcohol is usually employed in the extraction of the soluble carbohydrates from plant tissues. Constituents other than the mono- and di-saccharides were extracted with the alcohol, the total quantity extracted amounting to 43.82 per cent. of the dry weight of the whole peel from fresh green lemons, and 40 per cent. of that from mature lemons from packing-house storage (table I). The total water-soluble sugars (calculated as glucose) in the alcoholic extract composed, on the average, 26.41 per cent. of the dry weight and 60.29 per cent. of the alcohol-soluble solids of the green fruit peel. Similar values from the mature fruit samples were significantly lower than those from the green fruit, as the former had been in storage for five weeks and the soluble sugars in the peel had diminished during this period.

Differences in the amounts of soluble sugars in the various peel samples are related to the total amount of material in the alcohol-soluble fraction. The nonreducing sugars were inverted with a highly active invertase solution and expressed as sucrose. Apparently, the reducing sugars are the predominating water-soluble carbohydrates of the peel. The alcoholic extract from both the green and the mature samples contained, therefore, approximately 40 per cent. of its solids in a form other than reducing sugars and sucrose. The alcohol-soluble solids contained substances which gave a small amount of CO_2 (mean, 0.559 per cent. in the green samples and 0.450 per cent. in the mature samples) on hydrolysis with 12 per cent. hydrochloric acid.

The percentages of water-soluble sugars (dry-weight basis) in the 80 per cent. alcoholic extract of the lemon peel albedo (table II) were relatively higher than those of the whole peel (table I). The percentages of total sugars (dry-weight basis) in the albedo of green peel (mean, 36.21 per cent.) were not significantly different from those of peel from mature lemons that ripened on the tree (mean, 36.06 per cent.). There is a difference in these samples, however, in the proportion of total alcoholic extractives that are sugars, total soluble sugars constituting 76.60 per cent. of the extractives of the green samples and 96.80 per cent. of those of the mature samples. It is worthy of note that in the fully mature, tree-ripened lemons the alcohol-soluble substances in the albedo were composed mostly of soluble sugars. The data are in accord with those of Bartholomew and Robbins (**1**), who found that in albedo samples from mature lemons water-soluble sugars constituted the total substances extracted with alcohol.

Alcohol-insoluble solids of whole peel and albedo

The alcohol-insoluble solids of lemon peel are composed chiefly of the structural constituents of the cells. As there is no starch in the peel, this fraction is composed of carbohydrates of the cellulose, hemicellulose, and pectin types, rather than of temporary reserve materials. This fraction is excellently suited for a study of these structural constituents because it is free of all soluble carbohydrates and pigments, and of a considerable portion of the minerals.

The degree of efficiency with which alcohol separates the pectins of lemon peel is shown in tables I and II. The CO_2 produced by a plant material on hydrolysis with 12 per cent. HCl is quantitatively related to the amount of uronic acid (**13**). The uronic acids yield 22.50 per cent. CO_2. Since pectin is chiefly composed of uronic acids, the yield of CO_2 by the fractions should give a fair approximation of the pectin content. Pectin is relatively insoluble in alcohol; therefore, most of the CO_2-yielding substances should occur in the alcohol-insoluble-solids fraction. In the whole peel from green fruit, this fraction yielded an average of 8.47 per cent. of its weight as CO_2. The whole peel from mature fruit and the albedo from both green and mature fruit yielded about the same amount of CO_2 from the alcohol-insoluble solids. On the assumption that all of the CO_2 in the alcohol-insoluble fraction is derived from the pectin, the percentage of pectin (as calcium pectate) in this fraction can be estimated by the simple calculation $\frac{8.47 \times 100}{17.40}$, where 17.40 is the amount of CO_2 determined on highly purified calcium pectate.

The alcohol-insoluble solids of whole peel from green fruits contained an average of 5.59 per cent. methoxyl. The whole peel from mature fruit and the albedo from green fruit contained about the same amount of methoxyl in the alcohol-insoluble solids. Since this value is easily determined by saponification with NaOH, a quick method is available for the approximate determination of pectin. The uronic acid carboxyl groups of pectin are free for salt formation or esterification, but the hemicelluloses do not exhibit acidic properties when treated with alkali at room temperatures. The pectin content of a plant tissue, calculated from the methoxyl values, depends, of course, on the methoxyl content of pure pectin. Since the methoxyl content of pectin varies with the source, method of preparation, etc., an experimental factor has to be determined from a pure pectin prepared by a definite procedure.

Pectin content of the peel

Pectin is a carbohydrate complex composed of several polysaccharides such as araban, galactan, and polygalacturonic acid. The composition of pectin, as well as the quantity, is markedly affected by the methods of extraction. For example, the yield (**15**, **16**) of pectin from citrus albedo depends upon the pH at which the pectin is extracted, as well as upon the

TABLE III

WATER-SOLUBLE AND AMMONIUM-CITRATE-SOLUBLE PECTIN OF THE ALCOHOL-INSOLUBLE SOLIDS OF WHOLE LEMON PEEL, DETERMINED AS CALCIUM PECTATE*

Sample	Water-soluble pectin as calcium pectate				Ammonium-citrate-soluble pectin as calcium pectate				Total carbon dioxide**				Total calcium pectate	
	Total**	Constituents†			Total**	Constituents†			In A.I.S.	In calcium pectate	In residue	Undetermined	Determined**	Calculated from CO_2††
		CO_2	Ca	Furfural		CO_2	Ca	Furfural						
	%	%	%	%	%	%	%	%	%	%	%	%	%	%
Light-green lemons, 2½ inches in diameter														
A	21.08	18.13	8.57	19.63	14.39	17.10	8.01	19.70	8.16	6.28	1.10	0.78	35.47	76.98
B	20.27	17.98	8.64	19.80	14.92	18.55	7.71	19.55	8.48	6.41	0.90	1.17	35.19	75.59
C	20.40	17.71	8.22	19.22	14.46	17.55	7.96	19.60	8.63	6.15	1.23	1.25	34.86	71.26
D	20.34	18.08	8.32	19.66	14.95	17.61	7.81	19.76	8.50	6.31	1.27	0.92	35.29	74.24
Mean	20.52	17.98	8.44	19.58	14.68	17.70	7.87	19.65	8.44	6.29	1.12	1.03	35.20	74.52
Mature lemons from packing-house storage														
A	25.53	17.66	8.17	18.75	10.25	17.48	8.04	19.22	8.04	6.30	0.95	0.79	35.78	78.36
B	25.53	18.14	8.57	20.15	10.42	17.66	8.01	19.78	7.77	6.47	1.01	0.29	35.95	83.27
C	25.70	17.78	8.42	19.60	9.34	18.14	8.51	20.10	7.96	6.26	0.74	0.96	35.04	78.64
D	25.17	17.94	8.67	18.58	10.07	17.48	8.48	20.10	8.04	6.28	1.09	0.67	35.24	78.11
E	25.73	17.83	8.76	20.88	9.11	18.27	8.38	19.20	7.68	6.25	0.84	0.59	34.84	81.38
Mean	25.53	17.87	8.52	19.59	9.84	17.81	8.28	19.68	7.90	6.31	0.93	0.66	35.37	79.95

* Calcium pectate samples were dried to constant weight at 105° C.
** Values expressed as percentages of alcohol-insoluble solids.
† Values expressed as percentages of calcium pectate.
†† Values expressed as percentages of total carbon dioxide of the alcohol-insoluble solids.

duration and temperature of the extraction. A standard procedure for the extraction of pectin must be adhered to if reliable results are to be expected. Abundant evidence is available to show that pectins of fruits are not present in the cells and cell walls as fixed and permanent entities, but exist in labile forms which may be transformed with the growth and maturity of the fruit. The composition of pectic acid varies with the treatment received during its preparation.

Since pectic acid is composed of polygalacturonic acids combined with various amounts of l-arabinose and d-galactose, repeated purification of the material results in a gradual decrease in l-arabinose and d-galactose, with an ultimate approach to pure galacturonic acid. This makes the quantitative separation and determination of pectin, hemicellulose, cellulose, and lignin a rather complicated and difficult procedure. The polyuronides decompose slowly on hydrolysis with hydrochloric or sulphuric acid below a concentration of 5 per cent., and the free uronic acids (**14**) that are formed in the heating are readily decarboxylated. According to LINK and NIEMANN (**14**), this destruction of the uronic acids is a source of error in the total reducing power after an acid hydrolysis of the hemicellulose fraction. The magnitude of the error obviously depends on the amount of uronic acids in the hemicellulose fraction. To estimate the hemicellulose by the amount of reducing substances produced on hydrolysis with either weak acid or alkali is of little value unless the pectins and polyuronides are considered, for the uronic acids are present chiefly in the pectins and hemicelluloses.

As shown in table III, the pectins were extracted from the alcohol-insoluble solids of whole lemon peel with water and ammonium citrate. The values were determined as calcium pectate and reported as percentages of the alcohol-insoluble solids. The calcium pectates from the water extracts of the green and mature fruit samples averaged 20.52 per cent. and 25.53 per cent., respectively. The calcium pectates from the weak (1 per cent.) ammonium citrate extracts of the same samples, subsequent to water extraction, were unexpectedly low, amounting to 14.68 per cent. and 9.84 per cent., respectively. The total pectin extracted (sum of water-soluble and ammonium citrate-soluble pectin) was 35.20 per cent. for the green fruit and 35. 37 per cent. for the mature fruit. As would be expected, the mature fruit from the packing-house storage contained more water-soluble pectin than the green fruit, but the total pectin extracted (as calcium pectate) was approximately the same in both types of samples.

In addition to the pectin, the ammonium citrate solution extracts organic materials that are closely associated with the pectins in physical properties. These substances are probably celluloses and hemicelluloses. The lengthy procedure used for purifying the pectins and calcium pectates of this fraction results in a low yield but a fairly satisfactory product.

The degree of purity of the calcium pectates can be ascertained from their respective carbon dioxide, calcium, and furfural values. The yields

of CO_2 from the calcium pectates are slightly higher than the 17.40 per cent. (mean) obtained by JOSEPH and BRYANT (**11**) from calcium pectates from lemon pectin. The calcium contents are higher than those usually reported for calcium pectate from citrus (**7**) and from apple pectins (**5**), and they are higher than would be expected from the CO_2 content—that is, on the basis that calcium pectate should contain 7.45 per cent. calcium. GADDUM (**7**) showed that the calcium content of calcium pectates prepared from pectins which had been repeatedly precipitated with alcohol approached a constant value of 7.5 per cent. In his water-soluble albedo pectins the initial high calcium values were associated with the presence of high ash values. In the present studies the purity of the calcium pectates can be shown by the fact that the alkalinity of the ash is in good agreement with the percentages of calcium (table VI). The furfural content of the various calcium pectates is in accord with values reported by other investigators (**17**) on pectins from other sources, and especially do they agree with the results of BRYANT, PALMER, and JOSEPH (**4**) on lemon pectin (NATIONAL FORMULARY, ed. VIII) containing a high content of galacturonic acid (87.6 per cent.). No claim is made that pectic acid can be freed of arabinose and galactose by precipitation as the insoluble calcium pectate. The complete removal of galactose and arabinose from pectin by hydrolysis (as by hydrochloric acid in methyl alcohol) results in a degraded form of pectic acid. On the basis of the present analyses, the calcium pectate values given (tables III and IV) represent, to a considerable degree of accuracy, the pectin content of these extracts.

The amount of carbon dioxide in the total calcium pectate reported in green and mature lemon peel (table III) was 6.29 per cent. and 6.31 per cent., respectively, of the alcohol-insoluble solids. These percentages, when divided by the total carbon dioxide derived from the alcohol-insoluble solids, give the percentages of the total carbon dioxide obtained from the calcium pectate. They are expressed (in table III) as the calcium pectate calculated from the carbon dioxide. In other words, the amount of calcium pectate determined on the two different samples averaged 74.52 per cent. and 79.95 per cent. of the pectin calculated from the total carbon dioxide of the alcohol-insoluble solids. It can be seen that the sum of the carbon dioxide derived from the calcium pectate and residue does not account for the total amount in the alcohol-insoluble solids. The undetermined fraction represents the carbon dioxide lost in the isolation and purification of the pectin.

The data of table IV show the water-soluble and HCl-soluble pectins from the alcohol-insoluble solids of the albedo. The albedo used in these determinations was taken from different fruit samples than those used for similar data on the whole peel (table III). The water-soluble pectin extracted from the green-peel albedo was surprisingly high (mean, 26.63 per cent.). The only explanation suggested for these results is that the pectin was hydrolyzed when the albedo was initially heated to boiling in 80 per cent. alcohol to inactivate the enzymes. Another distinctive feature of the

TABLE IV

WATER-SOLUBLE AND ACID (HCl)-SOLUBLE PECTIN OF ALCOHOL-INSOLUBLE SOLIDS OF LEMON PEEL ALBEDO, DETERMINED AS CALCIUM PECTATE*

SAMPLE	TOTAL CO_2 IN A.I.S.**	WATER-SOLUBLE PECTIN AS CALCIUM PECTATE				ACID (HCl)-SOLUBLE PECTIN AS CALCIUM PECTATE				TOTAL CALCIUM PECTATE**
		TOTAL**	CONSTITUENTS†			TOTAL**	CONSTITUENTS†			
			CO_2	Ca	FURFURAL		CO_2	Ca	FURFURAL	
%	%	%	%	%	%	%	%	%	%	%
IMMATURE GREEN LEMONS, 2¼ INCHES IN DIAMETER										
1	8.01	26.67	18.25	8.84	19.50	8.56	18.03	7.99	19.78	35.23
2	8.12	26.10	17.85	8.62	19.85	8.89	18.72	8.40	20.51	34.99
3	8.01	26.92	17.62	8.74	19.13	8.20	19.43	8.03	20.19	35.12
4	8.12	27.16	17.65	8.85	19.66	8.90	17.47	7.67	19.50	36.06
5	8.07	26.30	18.22	8.66	20.22	9.16	16.80	7.92	19.76	35.46
Mean	8.07	26.63	17.92	8.74	19.67	8.74	18.09	8.00	19.95	35.37
MATURE, TREE-RIPENED LEMONS, 2¼ INCHES IN DIAMETER										
1	8.66	30.68	17.02	8.83	18.58	8.88	18.03	8.37	20.90	39.56
2	8.82	29.30	18.64	8.52	18.56	10.24	17.81	8.05	19.22	39.54
3	8.28	30.66	18.04	8 68	19.65	9.20	17.25	7.66	20.10	39.86
4	8.39	29.30	18.82	8.78	20.15	9.30	17.40	8.26	.....	38.60
5	8.82	29.10	18.13	8.59	20.88	9.76	17.85	8.01	.....	38.86
Mean	8.59	29.81	18.13	8.68	19.56	9.47	17.67	8.07	20.07	39.28

* Calcium pectate samples were dried to constant weight at 105° C.
** Values expressed as percentages of alcohol-insoluble solids.
† Values expressed as percentages of calcium pectate.

TABLE V

RELATION BETWEEN THE TOTAL CALCIUM PECTATE EXTRACTED AND THE PECTIN EQUIVALENT CALCULATED FROM THE PERCENTAGES OF CARBON DIOXIDE AND METHOXYL IN THE ALCOHOL-INSOLUBLE SOLIDS OF WHOLE LEMON PEEL

SAMPLE	ALCOHOL-INSOLUBLE SOLIDS*		PECTIN EQUIVALENT*			TOTAL CALCIUM PECTATE DETERMINED*	FRACTION OF TOTAL PECTIN AS CALCIUM PECTATE†
	TOTAL CARBON DIOXIDE	TOTAL METHOXYL	CARBON DIOXIDE ($CO_2 \times 5.75$)	METHOXYL ($OCH_3 \times 9.09$)	MEAN		
	%	%	%	%	%	%	%
LIGHT-GREEN LEMONS, 2¼ INCHES IN DIAMETER							
A	8.16	5.70	46.92	51.81	49.37	35.47	71.85
B	8.48	5.48	48.76	49.81	49.29	35.19	71.39
C	8.63	5.62	49.62	51.09	50.36	34.86	69.22
D	8.59	5.55	49.39	50.45	49.92	35.29	70.69
Mean	8.47	5.59	48.67	50.79	49.74	35.20	70.79
MATURE LEMONS FROM PACKING-HOUSE STORAGE							
A	8.04	5.00	46.23	45.45	45.84	35.78	78.05
B	7.77	5.07	44.68	46.09	45.39	35.95	79.20
C	7.96	5.07	45.77	46.09	45.93	35.04	76.29
D	8.04	5.07	46.23	46.09	46.16	35.24	76.34
E	7.68	5.22	44.16	47.45	45.81	34.84	76.05
Mean	7.90	5.09	45.41	46.23	45.83	35.37	77.19

* Values expressed as percentages of alcohol-insoluble solids.
† Values expressed as percentages of mean pectin equivalent.

data of table IV is the high water-soluble and total pectin values of the albedo from the mature lemons. The yield of calcium pectate from these mature fruits was higher than from any of the other samples studied. These lemons were more than a year old and had thoroughly ripened on the tree. The pectin was much easier to remove from the tissue by the extractants. The acid-soluble pectin (table IV) was extracted from the tissue with hydrochloric acid at pH 2.50. After the water-soluble pectin was extracted from the alcohol-insoluble solids, the residue was hydrolyzed with acid under conditions that freed the pectin from the cellulose material and at the same time caused as little destruction of the pectin as possible.

By the use of appropriate factors, the pectin content of whole peel calculated from either the carbon dioxide or the methoxyl values of the alcohol-insoluble solids gives a fair approximation of the total pectin of this material (table V). Although the carbon dioxide values are specific for a definite class of organic compounds (uronic acids), the methoxyl groups are widely distributed in plant substances such as alkaloids and various constituents of volatile oils, and in all plant materials containing lignin. PHILLIPS, GOSS, and BROWNE (**18**) determined the carbon dioxide and methoxyl content of 23 different plant materials and concluded that no correlation was evident between the percentage of uronic acid anhydride and the methoxyl content of these substances. In lemon peel the situation is quite different. The carbon dioxide and methoxyl contents of the alcohol-insoluble solids are highly correlated with the pectin content of the peel (table V). Lemon peel contains very little if any lignin; consequently, the alcohol-insoluble solids are composed chiefly of cellulose, hemicellulose, and pectin. Substances other than pectin that might contribute to the methoxyl values were extracted with the 80 per cent. alcohol. The fact that there was no significant difference between the methoxyl values, as determined by the saponification and Zeisel methods, indicates that the methoxyl groups in the alcohol-insoluble solids are derived from the pectin. If this fraction of the peel contained appreciable quantities of lignified material, the Zeisel methoxyl values would certainly be in excess of the amount equivalent to the pectin content, for the Zeisel method determines the total methoxyl groups but the saponification procedure determines only the methoxyl groups combined as an ester.

The calculation of the pectin content of a plant material from the CO_2 values does not always indicate that an equivalent quantity of pectin can be isolated. If a portion of the uronides is combined with substances other than the pectins, a quantitative isolation of pectin equivalent to the CO_2 content is very difficult. With many plant tissues, the calcium pectate and uronic acid methods are not completely interchangeable. The calcium salt of any polysaccharide containing an acid group may occur as an impurity in the calcium pectate. Such impurities are difficult to separate from the calcium pectate even on purification by dissolving and reprecipitation. If the pectin content of a plant material is to be reported as calcium pectate,

the values should be accompanied by the percentages of carbon dioxide and calcium. As noted by KERTESZ (**12**), certain calcium pectate figures which have been reported in the literature are obviously not a true measure of the pectin content of the tissue studied. By applying tests for the criteria of purity, the calcium pectate determined on a substance may be made a fair measure of the true pectin content of the material.

The fraction of the total pectin (table V) experimentally determined is obtained by expressing the total calcium pectate values as percentages of the mean pectin equivalent. On this basis, the sum of the calcium pectate from the water and ammonium citrate extracts amounted to 70.79 per cent. and 77.19 per cent. in green and mature samples, respectively.

CALCIUM CONTENT AND ALKALINITY OF THE ASH OF CALCIUM PECTATE OF THE ALBEDO

The purity of calcium pectate in lemon peel albedo may be estimated by determining the relation of the calcium to the alkalinity of the ash. If the calcium content equivalent to the alkalinity of the ash is greater than the calcium experimentally determined, the system indicates that a cation other than calcium is combined with the pectin. If the reverse is true, the system indicates than an inorganic anion is combined with a portion of the calcium.

TABLE VI

RELATION OF THE CALCIUM CONTENT OF LEMON PEEL ALBEDO TO THE ALKALINITY OF THE ASH OF CALCIUM PECTATE*

EXPERIMENT NO. AND MATERIAL		ALKALINITY OF CALCIUM PECTATE ASH	CALCIUM EQUIVALENT OF ALKALINITY	CALCIUM DETERMINED		CALCIUM MINUS ALKALINITY OF ASH
		m.e./gm.	%	*m.e./gm.*	%	*m.e./gm.*
1.	Mature albedo	4.41	8.82	4.66	9.32	0.25
2.	Mature albedo	4.52	9.04	4.73	9.46	0.21
3.	Mature albedo	4.59	9.18	4.67	9.34	0.08
4.	Mature albedo	4.55	9.10	4.64	9.28	0.09
5.	Mature albedo	4.58	9.16	4.73	9.46	0.15
6.	Mature albedo	4.38	8.76	4.66	9.32	0.28
7.	Mature albedo	4.41	8.82	4.70	9.40	0.29
8.	Mature albedo	4.39	8.78	4.60	9.20	0.21
9.	Mature albedo	4.32	8.64	4.58	9.16	0.26
10.	Mature albedo	4.16	8.32	4.43	8.86	0.27
11.	Mature albedo	4.26	8.52	4.52	9.04	0.26
12.	Mature albedo	4.11	8.22	4.51	9.02	0.40
13.	Mature albedo	4.06	8.12	4.53	9.06	0.47
14.	Mature albedo	4.50	9.00	4.56	9.12	0.06
15.	Mature albedo	4.57	9.14	4.63	9.26	0.06
16.	Green albedo	4.59	9.18	4.80	9.60	0.21
17.	Green albedo	4.64	9.28	4.83	9.66	0.19
18.	Green albedo	4.40	8.80	4.06	8.12	−0.34
19.	Green albedo	4.36	8.72	4.18	8.36	−0.18
20.	Green albedo	4.58	9.16	4.78	9.56	0.20
21.	Green albedo	4.51	9.02	4.76	9.52	0.25
Mean		4.42	8.85	4.60	9.20	0.22

* All calcium pectate samples were dried to constant weight at 125° C.

Results (table VI) show that the calcium of the albedo averages 0.22 m.e./gm. of calcium greater than the alkalinity of the ash. On the assumption that for pure calcium pectate the alkalinity of the ash is equivalent to the amount of calcium determined, a correction should therefore be made for the excess calcium. The data reported were obtained on purified calcium pectate prepared from water-soluble pectin.

The calcium values (mean, 9.20 per cent.) of these samples (table VI) are higher than those usually reported in the literature for calcium pectate. This is partly due to the fact that the samples were dried at 125° C instead of at 105° C. These apparently high calcium values are in very close agreement with the data of JOSEPH and BRYANT (**11**), who, on a series of calcium pectates from lemon pectin, obtained an average of 9.3 per cent. calcium on samples dried at 135° C. These workers also found that a higher temperature than 105° C is needed to free calcium pectate of occluded moisture. The present writers obtained from 3 to 5 per cent. more moisture from calcium pectate at 125° C than at 105° C, with samples weighing 100 to 150 mg. The calcium pectate samples in these studies were dried in thin sheets. If this material is not thinly spread, the rapid drying of the surface produces case hardening, which makes it very difficult to dry the calcium pectate completely at 105° C.

The uniformity of the calcium content of calcium pectate is evidence that the pectin precipitated by the calcium ion is a chemical substance of considerable uniformity. The claim is frequently and justly made that the calcium pectate method determines the pectin and very few of its decomposition products. The close association of the pectin with the other cell-wall constituents is such that the extraction of the pectin by chemical methods would result in a calcium pectate of variable composition, especially if the extraction processes are prolonged. The calcium pectate method can be used to estimate the pectin content of citrus peel if proper care is used in extracting the pectin from the tissue. In the present study the calcium in calcium pectate from the water-soluble pectin was higher than similar values from the acid-soluble extracted pectin (table IV).

RELATION OF URONIC ACID ANHYDRIDE TO FURFURAL CONTENT OF ALCOHOL-INSOLUBLE SOLIDS OF THE ALBEDO

It has been well established that pentose sugars, and also the dicarboxylic acids from the hexoses, yield the furane system under the proper conditions of experimentation. The yield of furfural from lemon albedo is derived chiefly from the pentoses of the pentosans and from the uronic acids and pentoses of the pectins. In plant materials the uronic acids occur chiefly in the pectins and hemicelluloses. In lemon albedo, however, the total uronic acid content is highly and positively correlated with the total amount of pectin isolated. The furfural obtained by the difference between the total furfural of the albedo and the amount equivalent to the uronic acids is ordinarily used to calculate the pentosans. On hydrolysis with 12

TABLE VII

RELATION OF URONIC ACID ANHYDRIDE TO FURFURAL CONTENT OF ALCOHOL-INSOLUBLE SOLIDS OF LEMON PEEL ALBEDO

Sample	Alcohol-insoluble solids of albedo*							Furfural (dry wt.) in:		
	Carbon dioxide	Uronic acid (galacturonic) anhydride		Furfural equivalent to uronic acid anhydride	Total furfural (A.I.S. basis)	Furfural from substances other than uronic acid anhydride	Pentosans equivalent to furfural from substances other than uronic acids (×1.72)	Whole albedo	Alcohol-insoluble solids	Alcohol-soluble solids
		$CO_2 \times 4$†	Equivalent to methoxyl							
	%	%	%	%	%	%	%	%	%	%
Immature green lemons, 2¼ inches in diameter										
1	8.01	32.04	28.68	7.52	15.46	7.94	13.66	9.76	8.30	1.46
2	8.12	32.48	30.03	7.62	15.36	7.74	13.31	10.04	8.19	1.85
3	8.01	32.04	29.96	7.52	15.36	7.84	13.48	10.06	7.98	2.08
4	8.12	32.48	28.57	7.62	15.62	8.00	13.76	10.38	8.02	2.36
5	8.07	32.28	29.27	7.57	15.90	8.33	14.33	9.74	8.33	1.41
Mean	8.07	32.26	29.30	7.57	15.54	7.97	13.71	9.99	8.16	1.83
Mature, tree-ripened lemons										
1	8.66	34.64	33.03	8.14	16.21	8.07	13.88	11.40	9.85	1.55
2	8.82	35.28	33.32	8.29	16.86	8.57	14.74	11.43	10.75	0.68
3	8.28	33.12	33.91	7.78	16.40	8.62	14.83	10.80	10.23	0.57
4	8.39	33.56	33.32	7.88	16.03	8.15	14.02	11.33	10.20	1.13
5	8.82	35.28	33.32	8.26	16.04	7.78	13.38	10.80	10.13	0.67
Mean	8.59	34.38	33.38	8.07	16.31	8.24	14.17	11.15	10.23	0.92

* Values expressed as percentages of alcohol-insoluble solids.

† The factor "4" is obtained by dividing the molecular weight of galacturonic acid anhydride (176) by the molecular weight of carbon dioxide (44).

per cent. hydrochloric acid, uronic acids yield a quantitative amount of CO_2, but the furfural produced is less than the theoretical amount.

LEFEVRE and TOLLENS (**13**) found that glucuronic acid anhydride, on hydrolysis with 12 per cent. hydrochloric acid, yielded an average of 19.11 per cent. of its weight as furfural; this resulted in a ratio of furfural to glucuronic acid anhydride of 1 to 5.23. Similar determinations were made by NORRIS and RESCH (**17**), who found that glucuronic acid anhydride (euxanthic acid) yielded 21.48 per cent. of its weight as furfural, or a ratio of furfural to glucuronic acid anhydride of 1 to 4.66. With galacturonic acid anhydride, the yield of furfural (**17**) was 23.50 per cent., and the resulting ratio of furfural to galacturonic acid anhydride was 1 to 4.26. The percentage of furfural derived from galacturonic acid anhydride is equal to the latter divided by 4.26. This relationship gives a close approximation of the amount of furfural yielded by the uronic acids of the alcohol-insoluble solids. Substances, other than uronic acids and polyuronides, that yield furfural, such as pentoses and hexoses, are not present in the alcohol-insoluble solids. The difference between the percentage of total furfural and the percentage of furfural produced by the galacturonic acid when multiplied by 1.72 gives the percentage of pentosans.

The data of table VII show the relation of the amount of uronic acid anhydride to the amount of furfural in the alcohol-insoluble solids. The uronic acid anhydride determinations were calculated from both the carbon dioxide and the saponification values of the alcohol-insoluble solids. The values calculated from the percentages of carbon dioxide are significantly higher than those calculated from the saponification values. The lower values can be partially explained by the fact that some of the pectin is combined with cellulose as protopectin, through the carboxyl groups; this signifies that not all of the carboxyl groups are completely methoxylated. With the exception of furfural in the alcohol-soluble solids, the constituents reported are slightly higher in mature lemons.

The values for the uronic acid anhydride (table VII) were obtained by multiplying the percentages of CO_2 in the alcohol-insoluble solids by the factor 4. The furfural equivalent to the uronic acid anhydride is equal to approximately 50 per cent. of the total furfural of the alcohol-insoluble fraction. The percentages of furfural derived from substances other than uronic acid, multiplied by the factor 1.72, give the pentosan values of the alcohol-insoluble solids. The pentosan values can be expressed as pentoses if the furfural values are multiplied by the factor 1.95 instead of 1.72. The mean pentose content of the albedo from mature lemons would be $8.04 \times 1.95 = 15.68$ per cent. The total furfural yielded by the alcohol-insoluble fraction of the albedo averaged 15.54 per cent. and 16.31 per cent. in the green and mature lemons, respectively. For mature lemons, the mean value agrees satisfactorily with the data previously reported by SINCLAIR and BARTHOLOMEW (**20**).

The difference between the percentage of furfural yielded by the whole albedo and that yielded by the alcohol-insoluble solids (dry-weight basis) gives the percentage of furfural yielded by the alcohol-soluble fraction. As

TABLE VIII

RESIDUE AFTER EXTRACTION OF PECTIN FROM ALCOHOL-INSOLUBLE SOLIDS OF LEMON PEEL

METHOD OF PECTIN EXTRACTION	EXPERIMENT NO. AND MATERIAL	RESIDUE*		
		CELLULOSE AND HEMICELLULOSE FRACTION %	CARBON DIOXIDE %	FURFURAL %
H_2O + NH_4 CITRATE + NH_4OH	(1) MATURE PEEL	33.42	.....	.....
	(2) MATURE PEEL	35.32	.....	.....
	(3) GREEN ALBEDO	36.96	0.33	.....
Mean		35.23	0.33	.....
H_2O + NH_4 CITRATE	(1) MATURE PEEL	40 93	.....	.....
	(2) GREEN PEEL	44.16	1.25	.....
	(3) GREEN PEEL	43.06	1.25	.....
	(4) GREEN ALBEDO	46.91	1.10	.....
	(5) GREEN ALBEDO	45.98	0.90	.....
	(6) MATURE ALBEDO	42.82	0.95	.....
	(7) MATURE ALBEDO	43.64	1.00	.....
	(8) MATURE ALBEDO	47.98	1.34	.....
	(9) MATURE ALBEDO	46.20	1.01	6.58
	(10) MATURE ALBEDO	41.82	0.74	6.45
	(11) MATURE ALBEDO	44.54	0.84	6.35
	(12) MATURE ALBEDO	42.70	0.85	5.73
	(13) MATURE ALBEDO	46.08	1.09	6.32
	(14) MATURE ALBEDO	48.04	1.37	6.42
Mean		44.63	1.05	6.31
H_2O + TARTARIC ACID	(1) MATURE ALBEDO	36.66	1.12	5.22
	(2) GREEN ALBEDO	41.36	1.41	.....
	(3) GREEN ALBEDO	44.00	.....	.....
	(4) GREEN ALBEDO	43.40	.....	.....
	(5) GREEN ALBEDO	39.40	.....	.....
	(6) GREEN ALBEDO	41.20	.....	.....
Mean		41.00	1.27	5.22
H_2O + TARTARIC ACID + NH_4OH	(1) GREEN ALBEDO	32.26	.....	.....
	(2) GREEN ALBEDO	37.82	.....	.....
Mean		35.04	.....	.....
H_2O + HCl + NH_4 CITRATE	(1) MATURE ALBEDO	36 60	0.77	.....
	(2) MATURE ALBEDO	36.20	0.77	.....
	(3) MATURE ALBEDO	34.32	0.78	.....
	(4) MATURE ALBEDO	33.24	0.69	.....
	(5) MATURE ALBEDO	32.10	0 65	.....
	(6) MATURE ALBEDO	31.60	0.60	.....
	(7) MATURE ALBEDO	28.46	0.65	.....
	(8) MATURE ALBEDO	28.72	0.69	.....
Mean		32.65	0.70	.....

* Values expressed as percentages of the alcohol-insoluble solids.

TABLE IX

RESULTS OF HYDROLYSIS OF THE CELLULOSE AND HEMICELLULOSE FRACTION OF LEMON PEEL ALBEDO WITH 2 PER CENT. HYDROCHLORIC ACID*

MATERIAL	TOTAL PECTIN EXTRACTED AS CALCIUM PECTATE†	RESIDUE AFTER PECTIN EXTRACTION	FRACTION		RESIDUE AFTER HYDROLYSIS WITH 2 PER CENT. HCl	CONSTITUENTS HYDROLYZED		
			DETERMINED	UNDETERMINED		TOTAL	REDUCING SUGARS AS GLUCOSE	NON REDUCING SUBSTANCES
	%	%	%	%	%	%	%	%
Green albedo	34.85	30.79	65.64	34.36	20.60	10.19	7.02	3.17
Mature albedo	40.58	31.20	71.78	28.22	20.63	10.57	7.04	3.53

* All values expressed as percentages of alcohol-insoluble solids.
† Pectin extracted from alcohol-insoluble solids with water, dilute HCl, ammonium citrate, and ammonium hydroxide.

the alcohol-soluble fraction is composed chiefly of soluble carbohydrates, the major portion of the furfural obtained from this fraction is probably derived from the hexose sugars (**22**) rather than from the polyuronides and pentose-containing materials.

Cellulose and hemicellulose fraction of whole peel and albedo

In addition to cellulose, plant cell walls usually contain lignin, hemicellulose, and pectin. Lemon albedo contains little if any lignin. The other three classes of constituents are closely related to each other, especially the hemicellulose and pectin fractions. In nonlignified plant tissues the pectins usually exist in greater abundance than the hemicelluloses. This is particularly true of lemon peel.

The material that remains after extraction of the pectins from the alcohol-insoluble solids is composed chiefly of cellulose and hemicellulose, which, in turn, contain a comparatively small amount of firmly bound pectin that is difficult to extract and determine quantitatively. As noted (table VIII), the percentages of this fraction determined on the different peel samples vary, the extent depending, of course, upon the methods used to extract the pectin. The extractants used to dissolve the pectin also dissolve various portions of the hemicellulose. The residue remaining after extraction of the pectin with hot water, ammonium citrate, and dilute ammonium hydroxide is much smaller (35.23 per cent.) than the amount obtained when the final extraction with ammonium hydroxide is omitted (44.63 per cent.). In addition to a small amount of pectin, ammonium hydroxide extracts hemicelluloses. Considerable amounts of hemicelluloses are dissolved by water and tartaric acid, leaving a residue of 41.00 per cent. The residue remaining from the water, tartaric acid, and ammonium hydroxide extracts was about the same as that from the water, ammonium citrate, and ammonium hydroxide extracts. All the residues tested contained carbon dioxide; this shows the presence of uronic acids. After thorough extraction of the alcohol-insoluble solids with these solvents, the residues still contained pectin or uronic acids combined with celluloses or hemicelluloses.

Further study of the cellulose and hemicellulose fraction was made by hydrolyzing the material with 2 per cent. hydrochloric acid under a reflux condenser. The results of these experiments are reported as percentages of alcohol-insoluble solids (table IX) in order that these data may be compared with data of other tables. The fraction of the alcohol-insoluble solids accounted for is the sum of the total pectin extracted as calcium pectate and the residue after pectin extraction. As already noted, the amount of calcium pectate obtained from the alcohol-insoluble solids of the mature fruit was higher than that obtained from the green-fruit samples. This means that the fraction determined was correspondingly higher for the alcohol-insoluble solids of the mature fruit. The values reported for the undetermined fraction represent the nonpectinous material (some of the hemicelluloses) ex-

tracted by the chemical agents along with the pectin. After the extraction of the pectin, the amount of residue (the cellulose and hemicellulose fraction) is approximately the same in both the green and the mature samples. If this cellulose and hemicellulose fraction is hydrolyzed with 2 per cent. hydrochloric acid, an insoluble residue remains that amounts to approximately 20 per cent. of the alcohol-insoluble solids. This insoluble fraction is soluble at room temperature in the cellulose solvent of zinc chloride-hydrochloric acid solution. There remains, however, a small quantity of brown sediment (less than 0.5 per cent. by weight) which is insoluble in this solvent. The total amount of material hydrolyzed by the 2 per cent. hydrochloric acid was 10.19 per cent. and 10.57 per cent. for the green and the mature samples, respectively. Of this amount 67.7 per cent., or an average of 7.03 per cent. of the alcohol-insoluble solids, was determined as glucose. The nonreducing substances extracted with 2 per cent. hydrochloric acid averaged 3.35 per cent.

The results reported (table IX) do not reveal all the information that is desired of the cellulose and hemicellulose fraction. A more detailed study of this fraction is under way. The results do show, however, that the nonpectinous fraction of the alcohol-insoluble solids of the lemon albedo is composed chiefly of a hemicellulose fraction obtained by hydrolysis with dilute hydrochloric acid, and of a more resistant fraction resembling cellulose and soluble in the cellulose solvent.

Comments

From a biochemical point of view, the results of the present study show the general relationships of the carbohydrate constituents of lemon peel. The carbohydrate reserve is definitely low, as shown by the absence of starch and dextrin. No substantial amount of evidence exists to prove that the hemicellulose fraction acts as a carbohydrate reserve. HARVEY (**10**), by extracting macerated whole lemons with various solvents, found that the ratio of insoluble solids to soluble solids decreased with the advance of maturity. In experiments on stored lemons, he found a steady decrease in the ratio to a minimum near the close of the storage period, followed, in general, by a slight increase. As most of the insoluble solids are cell-wall constituents, the ratios given for the lemons at different stages of maturity may have been reduced by the increase in soluble solids rather than by an absolute decrease in insoluble solids. Such would not be the case in the storage experiments. With respect to decrease in cell-wall constituents of the peel, the pectin and hemicelluloses are metabolized on breakdown of the fruit. Published investigations have established the fact that the rate of pectin decomposition is associated with the keeping qualities of fruits. In lemon fruit the soluble pectin increases as the fruit changes from green to mature, either on the tree or in storage. The rate of change from insoluble to soluble pectin is a function of the temperature. Total pectin (the

soluble and insoluble fractions) decreases if the storage period is prolonged or if the fruit remains on the tree an excessive period after maturity.

The accuracy of some of the methods used in this investigation is not of the highest order, but the procedures finally adopted proved to be the most satisfactory for the experimental conditions. The fact that the solubility of pectin depends upon pH, temperature, and time of extraction is sufficient evidence to show that a given procedure must be defined and strictly followed if one is to obtain comparable results. An analyst thoroughly familiar with the methods and material can determine the pectins in the alcohol-insoluble solids of lemon peel with an error of less than 5 per cent. The more empirical a method is, the more necessary it is to follow a standard procedure.

The methods used in this study for the extraction and determination of pectin are concerned chiefly with the total amount of pectin rather than with a product having the physical and chemical properties so essential for a commercial grade of pectin. No claim is made that these methods are suitable for the commercial extraction of pectin, but they are useful for the quantitative estimation of pectin on small laboratory samples. From a biochemical standpoint, the relation of the total pectin to the other carbohydrate constituents of the peel is of importance, because some of the pectin in the peel is closely associated with the cellulose and hemicellulose fraction. The methods that are used, therefore, not only must be quantitative, but must produce a product with the least possible degradation.

The physiological interpretation of chemical data determined on plant tissues depends to a marked degree on the terms in which the results are expressed. In this investigation, the mean total water-soluble sugars of the lemon peel samples ranged from 21.59 to 36.21 per cent. of the dry weight (tables I and II). The moisture content of the peel varies considerably, depending, of course, upon orchard conditions at the time the fruit is picked. With such large fluctuations in dry matter and moisture, percentages expressed on either the wet-weight or the dry-weight basis may not reveal the progressive changes in the actual amount of a given constituent during growth. In the carbohydrate fractions of plants, an increase in the actual amount of one constituent may so affect the dry weight as to cause an apparent, but not an actual, decrease in another constituent. These conditions influence the interpretation of carbohydrate analyses of vegetative tissues containing various forms of carbohydrates. For example, the water content of lemon fruits composes more than 70 per cent. of the total weight of the fruit, and a large portion of the total solids is water-soluble. Soluble constituents of the juice are usually reported as percentages of fresh weight of the juice. Ash constituents and soluble carbohydrates of the peel are usually reported as percentages of their dry weights. Since the alcohol-soluble material composes such a large amount of the dry weight of the peel, the calcium pectate and the uronic acid values are expressed as percentages of the alcohol-insoluble solids.

Summary

The whole peel and albedo of lemon fruits have been extracted with 80 per cent. ethyl alcohol and separated into two distinct fractions, the alcohol-soluble and alcohol-insoluble solids. The carbohydrate constituents of these two fractions have been investigated, and certain of their physical and chemical properties have been determined.

The soluble sugars from whole peel or albedo averaged 54.0 to 96.8 per cent. of the total alcoholic extractives, the proportion depending upon the maturity of the fruit. In addition to soluble sugars, the alcohol-soluble fraction contained substances such as essential oils, waxes, organic acids, and various concentrations of undetermined constituents. The alcohol-soluble fraction also contained a substance which liberated a small amount of carbon dioxide on boiling with 12 per cent. hydrochloric acid.

As the alcohol-insoluble solids contained the pectin, most of the carbon dioxide occurred in this fraction. The sum of the water-soluble and ammonium citrate-soluble pectin (as calcium pectate) of the whole peel amounted to 35.29 per cent. (average value) of the alcohol-insoluble solids. Similarly, the sum of water-soluble and acid-soluble pectin (as calcium pectate) of the albedo amounted to 37.33 per cent. (average value) of the alcohol-insoluble solids. These values are much lower than those for total pectin calculated from carbon dioxide and methoxyl values, respectively.

To establish criteria of purity, the calcium pectate values were accompanied by corresponding values for carbon dioxide, calcium, and furfural. The percentages of calcium in the calcium pectates were somewhat higher than the 7.50 per cent. usually reported for pure calcium pectate.

The furfural equivalent to the uronic acid anhydride was equal to approximately 50 per cent. of the total furfural of the alcohol-insoluble fraction. The furfural derived from substances other than uronic acids is reported as pentosans.

The residue that remained after extraction of the pectin from the alcohol-insoluble solids was composed of cellulose and hemicellulose and a comparatively small amount of firmly bound pectin that is difficult to extract and determine quantitatively.

The residue that remained after extraction of the pectin from the hemicellulose fraction with 2 per cent. hydrochloric acid for three hours amounted to approximately 20 per cent. of the alcohol-insoluble solids. This insoluble fraction is soluble at room temperature in the cellulose solvent, zinc chloride-hydrochloric acid solution.

The authors are grateful to Drs. Glenn H. Joseph and Edwin F. Bryant, Research Department, California Fruit Growers' Exchange, for their constructive criticism of the paper.

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THE EFFECT OF TEMPERATURE ON THE GROWTH AND COMPOSITION OF THE STUBBLE AND ROOTS OF PERENNIAL RYEGRASS[1]

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(WITH SIX FIGURES)

Received April 25, 1949

In a previous paper (**12**) it was reported that, when perennial ryegrass (*Lolium perenne* L.) was partly defoliated, the remaining plant parts underwent a progressive loss in soluble carbohydrates, namely sugars and fructosan, for a period of several weeks. This loss from the stubble and roots accompanied a rapid formation of new tissue above ground. When new top growth had produced sufficient photosynthetic area to manufacture its own organic matter, storage of soluble carbohydrates took place. When the percentages of the constituents were plotted against time as the abscissa, the soluble carbohydrates displayed a typical U-shaped curve. Many factors undoubtedly influence the slope and extent of this curve and some of these are being investigated. The present report concerns the relationship of temperature to the composition of ryegrass after cutting.

The earlier paper included a review of the literature pertinent to the subject of reserves. Since then, other reports concerned with the analyses of forage grasses have verified the fact that soluble carbohydrates and starch function as reserves. More complex carbohydrates and those more resistant to hydrolysis, are not so regarded. Some of these recent papers are by BENEDICT and BROWN (**2**), PHILLIPS and SMITH (**9**), MCILVANIE (**8**), WEINMANN and REINHOLD (**18**), and WEINMANN (**13, 14, 15, 16**). A review by Weinmann covers the general subject of reserves in grasses (**17**).

Reserves in grasses are closely connected with the resistance of the plants to high temperatures. JULANDER (**6**) showed that grasses withstood limited exposures to a temperature of 48° C (118.4° F) when they had been previously hardened to dry conditions and had not been severely clipped. Both of these last conditions were conducive to the accumulation of reserve carbohydrates. He also noted differences between species, Kentucky bluegrass dying sooner at this temperature than Bermuda grass and a number of range grasses. At lower temperatures, grasses will survive for longer periods. According to HARRISON (**5**), Kentucky bluegrass grew very little at a constant temperature of 100° F after having been defoliated, and died

[1] Contribution No. 93 of the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, State College, Penna., in cooperation with the northeastern states.

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in about six weeks. At 80° F top growth was rapid but no new roots were produced and shoots from the rhizomes appeared above ground only under conditions of low nitrogen nutrition. Although no analyses were made of these plants it is assumed that the rapid top growth, combined with frequent clipping, soon depleted the carbohydrate reserves and led to their exhaustion. At 60° F growth was more normal for the species. According to LOVVORN (**7**), when four species of grasses were cut frequently, the yields were lower at 80° than at 65° F. BROWN (**3**) grew four grasses at a number of constant temperature levels between 40° and 100° F. In some species the optimum temperature for top growth was not the same as the optimum for root and rhizome production. For three grasses having lower optimum temperatures (70–90° for herbage, 50–70° for roots and rhizomes), namely Kentucky bluegrass, Canada bluegrass and orchard grass, an increase in temperature was associated with increased fiber and decreased nitrogen-free extract contents in above-ground and below-ground parts. Protein was at a minimum at 60° or 70° and was greater at both lower and higher temperatures. In Bermuda grass, which had an optimum growth temperature of 100°, fiber and nitrogen-free extract increased with rising temperature, the former to reach a limit of increase at 70°, the latter at 100°. The protein minimum was at 80–90°, somewhat higher than with the other grass species. In other studies of grasses, composition was not reported (**4, 10, 11**).

No information seems to be available concerning the composition of ryegrass at various temperatures. As it has been successfully grown only in cool temperate regions, its optimum temperature for growth is probably low. The temperature range in the studies reported here covers the optimum for growth of this species.

Materials and methods

The objective of the experiment here reported was to determine the effects of air and soil temperatures on the reserves of the stubble and roots of perennial ryegrass following clipping. Four temperature conditions were provided by growing plants in chambers with artificial light of 525 foot-candlepower intensity at the ground level. Air and soil temperatures were maintained by a time-cycle control so that maximum temperatures prevailed from 10 A.M. to 2 P.M. and minimum temperatures from 10 P.M. to 2 A.M. each day with gradual changes between them. Four such alternating air and soil temperature ranges were used: (1) 50°–60°, (2) 60°–70°, (3) 70°–80°, and (4) 80°–90° F. A 14-hour day was provided throughout the experiment and adequate soil moisture was maintained at all times.

The ryegrass clone used in a previous trial (**12**) was increased vegetatively to provide uniform material. Individual tillers, trimmed to remove all roots and old leaf growth, were placed in tap water for several days until new roots formed, then five tillers were planted in each of a number of one-gallon glazed crocks. The soil consisted of equal parts of a fertile Hagers-

town silty clay loam and river bottom sand, the latter to improve the texture of the soil and to facilitate washing of the roots when the plants were taken up. After about three months' growth in the greenhouse at 70° F under a 16-hour day (provided by supplementary Mazda light), the plants were clipped to a height of 1.5 inches above ground and placed in the chambers. At the time of clipping six pots were selected at random and the plants were removed, washed, and were used for dry weight determination and for chemical analysis. The data obtained from these are referred to as the "original" dry weights and the "original" composition. From the remainder of the plants placed in the chambers, four pots, selected at random, were removed for weight measurements and for chemical analysis at stated times, namely, after 4, 9, 14, 21, 28 and 40 days of exposure to the various temperature conditions. The leaf growth above the 1.5 inch level is referred to as "tops" and the remainder, except for the roots, as "stubble." The stubble was composed almost entirely of leaf tissue, largely leaf sheaths since the blades were removed with the tops and the stems in vegetative tillers of this grass species are very small. The roots were trimmed off with shears after having been washed in cold tap water.

Two separate trials were conducted to provide replication of results. The plants used in the first trial were started in the greenhouse December 9, 1942, and allowed to grow until March 13 when they were all clipped and placed in the chambers at the same time. Plants used in the second trial, also grown in the greenhouse, were not all started on the same date, but rather at succeeding intervals so that each group would have the same growth period as the comparable group in the first trial. Thus these plants were ready to place in the chambers when space became available following the removal of plants of the first trial. With this arrangement in the second trial, the plants were not all moved into the chambers at the same time but they came out on nearly the same date. For this reason there were six "original" samples in the second trial (or replication) but only one in the first.

The tops, stubble, and roots were analyzed separately by methods described earlier (**12**).

Results

Preliminary analyses for soluble carbohydrates

A preliminary sampling of plants was made on March 2, 11 days before the plants of the first trial were clipped and moved to the chambers. Analyses were carried out immediately to determine whether the plants contained reserve carbohydrates in sufficient amount for the studies planned. The results of the analyses are presented (table I) as the average of two samples, each consisting of material from five pots. As previously noted, the leaves and roots furnish a large part of the dry matter of the plant but their soluble carbohydrate content is relatively low. While the stubble contains less than 30 per cent. of the total dry matter it is higher in sugars and

much higher in fructosan than are either leaves or roots. Thus the lower parts of the above-ground portion of the plant contain more carbohydrates than the upper parts, and clipping (or grazing) removes an increasing proportion of them as the defoliation approaches ground level. In these plants the storage of reserve carbohydrates was found to be sufficient for the purposes of the experiment as planned.

TABLE I

YIELD AND CARBOHYDRATE CONTENT OF PARTS OF RYEGRASS PLANTS ON MARCH 2.

PART OF PLANT	WEIGHT OF PART IN PERCENTAGE OF WHOLE PLANT	CARBOHYDRATES, IN PERCENTAGE OF DRY WEIGHT		
		REDUCING SUGARS	SUCROSE	FRUCTOSAN
Upper 2/3 of leaves	15.4	2.3	7.6	1.0
Lower 1/3 of leaves	12.6	3.0	7.0	4.6
Upper 1/2 of stubble	10.6	6.8	9.1	21.7
Lower 1/2 of stubble	18.4	3.1	7.8	26.3
Roots	43.0	1.0	4.4	2.7

GROWTH OF CLIPPED PLANTS IN CONTROL CHAMBERS

The amount of growth made by the plants in the chambers was measured by determining at each sampling date the fresh weights of the new

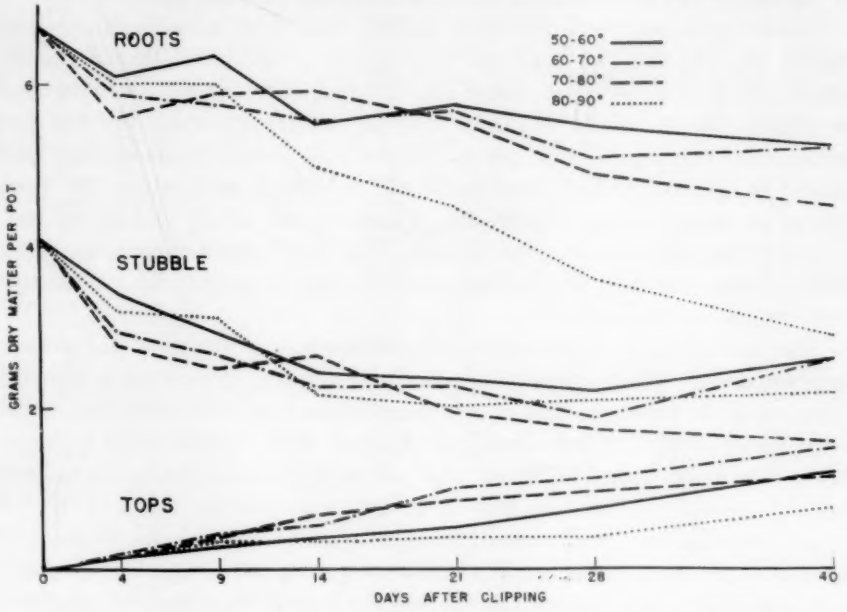

FIG. 1. Changes in the dry weights of the roots, stubble and tops of perennial ryegrass at four different temperature conditions.

top growth and the dry weights of top growth, stubble, and roots. The

variation in size of the plants on the days of clipping and the discrepancies between the two trials (or replications) made the dry weight changes brought about by temperature differences appear somewhat irregular. Using a mean of the "original dry weights" and the means of the two trials at the different sampling dates, the lines of figure 1 were drawn. It may be seen that there were some fluctuations, particularly at the early dates, but the general trends of dry matter yields are unmistakable.

New top growth appeared quickly following clipping and continued at all temperatures for the duration of the experiment. Differential rates of growth due to temperature were variable for the first two weeks but were more consistent for the later periods. The most top growth was produced by the series at 60–70°. While this series was somewhat higher in top growth yield than the average of the others at the three early sampling dates, it was highest of all series at the last three successive dates. On the other hand the 80–90° series was consistently lowest for the last four successive dates. The optimum temperature for this clone of ryegrass to produce foliage under the stated environmental conditions is therefore near 60–70°, with less and slower growth occurring at higher and lower ranges. The moisture content of the new foliage was also affected by temperature, and roughly correlated with yield. At the 40-day sampling the fresh top growth of the 60–70° series contained 18.6 per cent. dry matter, and that of the 80–90° series contained 23.5 per cent. dry matter.

While new tops were growing, stubble and roots were losing weight, rapidly for the first few days and less rapidly thereafter. In the stubble, which included new foliage below the 1.5 inch level as well as older leaf sheaths, a loss in weight was evident at all temperatures for the first two weeks after clipping. The plants at the two lowest temperatures soon ceased to lose weight but those at 70–80° continued to lose for the duration of the experiment. In the roots, a more or less steady loss in dry matter continued during the entire period. The two lower temperature series behaved alike but at higher temperatures the loss in weight was accentuated with the rise in temperature.

During the recovery period certain differences in the new leaf growth were apparent. At the time of the 21-day sampling, it was noted that the plants at 50–60° were second best in appearance and had leaves 2–5 inches long; those at 60–70° were healthy, vigorous, and of good color and had leaves 5–8 inches long; at 70–80° the plants had more spindly leaves and did not appear vigorous; at 80–90° growth was poorest, leaves were 1–5 inches long and very dark green, and roots were brown and discolored. At the end of 40 days the leaves at the lowest temperature were broadest and most vigorous in appearance; at 60–70° the leaves were slightly narrower and slightly darker green; at 70–80° the plants were still less vigorous; at 80–90° growth was stunted, the leaves were very dark green and had the lowest moisture content, and the roots were very dark brown—many may

have been dead. It was evident that the high air and soil temperatures were unfavorable for the growth of perennial ryegrass. New leaf growth was produced somewhat faster at 60–70°, but after forty days a more vigorous growth was apparent at 50–60° F.

It should be noted here that recovery in these plants grown under artificial conditions was not as rapid as in those previously reported under greenhouse conditions (**12**). In the greenhouse the roots and stubble, after initial weight fluctuations lasting for a few weeks, began to increase steadily in weight. During the 40-day recovery period under controlled environments as reported here, the roots and stubble did not attain weights comparable to those of plants sampled at the time of clipping and removal from the greenhouse. Lower light intensities and lower carbon dioxide concentrations in the chambers may have been factors limiting growth.

Composition changes of clipped plants in control chambers

On March 13 when all the plants included in the first trial were clipped, some of them were prepared for analysis. Two complete samples representing the tops from six plants and the stubble and roots from seven others were analyzed. In the second replication there were six "original" compositions at as many different dates with six pots contributing to each and their composition and weight were necessarily variable. For brevity only the mean values are presented here (table II).

TABLE II

Mean dry weights and composition of parts of plants when plants were moved into control chambers.

	First replication			Second replication		
	Leaves	Stubble	Roots	Leaves	Stubble	Roots
Dry weight per pot, grams	1.78	4.82	6.94	2.21	3.37	6.49
Sol. matter, %	35.0	18.8	14.7	31.4	14.3	15.0
Reducing Sugars, %	3.8	3.4	1.2	1.4	2.5	0.8
Sucrose, %	6.2	6.2	5.5	5.5	4.5	6.0
Fructosan, %	.....	27.5	4.9	.....	29.3*	5.8
Cellulose, %	.....	21.2	26.4	.....	.....	.....
Pentosan, %	.....	14.0	18.6	.....	.....	.....
Lignin, %	.....	3.6	10.3	.....	.....	.....
Sol. nitrogen, %	0.30	0.09	0.05	0.20	0.08	0.05
Insol. nitrogen, %	2.06	0.60	0.59	1.73	0.49**	0.59**
Total nitrogen, %	2.36	0.69	0.64	1.93	0.57	0.64
Sol. nitrogen, in % of total nitrogen	12.8	13.1	8.3	10.5	13.3	7.5
Crude ash, %	7.7	9.1	14.4	8.8	8.3	9.6
Calcium, %	0.47	0.19	0.30	.....	.....	.....
Phosphorus, %	0.13	0.10	0.08	.....	.....	.....

* Only four "original samples", two lost.
** Only five "original samples", one lost.

The changes in composition of the plants at the various temperatures

in the control chambers are next considered. Changes in the sucrose content are illustrated in figure 2. In the stubble these changes followed a U-shaped curve, sucrose reaching a low point about two weeks after clipping and rising thereafter. The lowest temperature series, 50–60°, showed con-

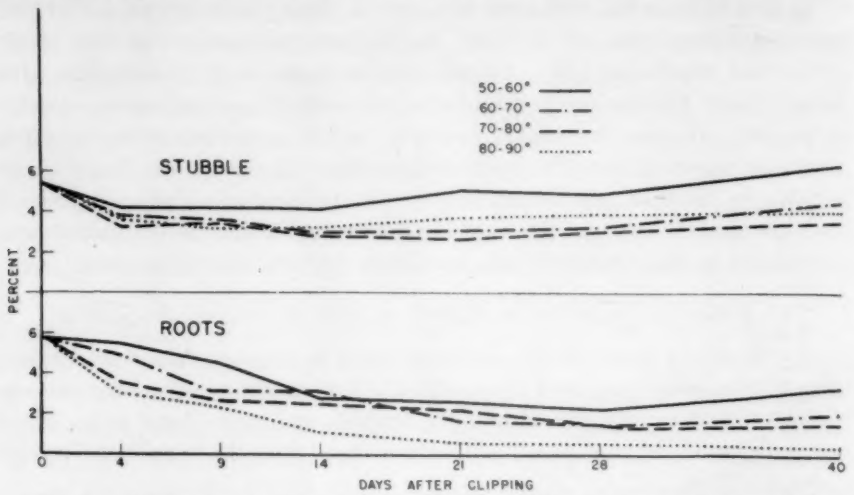

FIG. 2. Changes in the sucrose content of the stubble and roots of perennial ryegrass at four different temperature conditions.

siderable increases at the later periods, with sucrose exceeding its original concentration. In the roots the original concentration of sucrose was of the same order as that in the stubble but the initial fall was of longer duration, lasting about four weeks. Appreciable restorage occurred only in the 50–60° series, and to a lesser extent than in the stubble. The final sucrose concentration was definitely a function of temperature, the higher temperatures causing greater sucrose losses.

Reducing sugars were determined separately. Beginning in the stubble at 3 per cent. they showed a more gradual fall than did sucrose and showed little response to temperature, all four series ending in a range of 1.7 to 2.1 per cent. In the roots, reducing sugars were of minor importance, having an initial value of only 1 per cent. and fell uniformly to about 0.2 per cent. with no noticeable temperature effects. Because of the minor importance of reducing sugars in the roots and their failure to respond to temperature in either plant part, a graph for total sugars (reducing sugars plus sucrose) resembles that of sucrose but shows relatively smaller fluctuations.

The previously noted importance of fructosan as a reserve carbohydrate is confirmed by these experiments. In the stubble it amounted to more than one fourth of the dry matter at the time of clipping and a good part of this rapidly disappeared. The changes are illustrated in figure 3. As with sucrose, utilization was more rapid at higher temperatures and restorage took place only at the lower temperatures. In the roots, fructosan amounted

only to about 7 per cent. of the total dry matter. Its rate of utilization was similar to that in the stubble and, considering that the roots exceeded the stubble in total weight, its loss from the roots was considerable. The regularity of the loss of fructosan with rise in temperature was almost without exception, both in the stubble and in the roots.

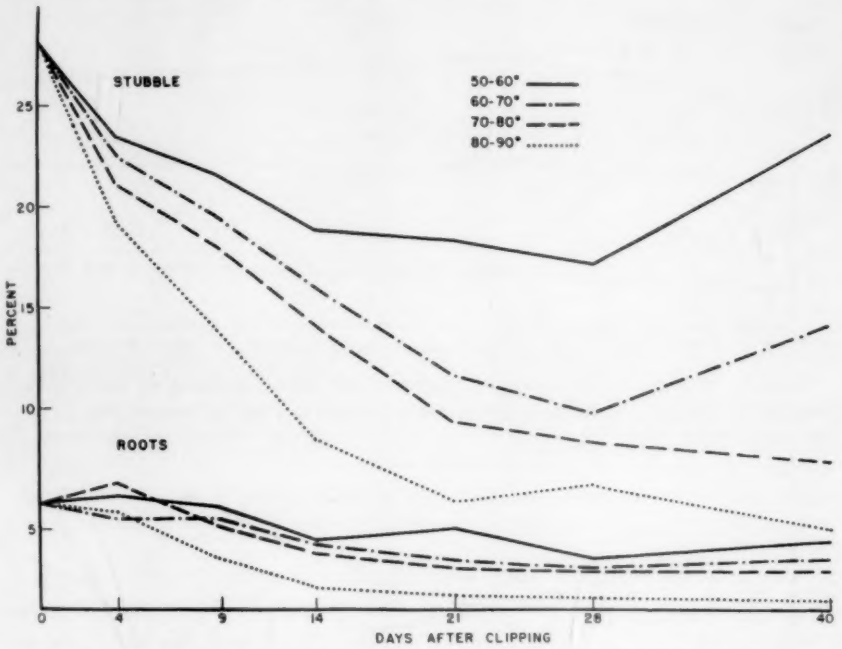

FIG. 3. Changes in the fructosan content of the stubble and roots of perennial ryegrass at four different temperature conditions.

On the other hand, the structural constituents, cellulose, lignin, and pentosan, did not undergo loss after clipping. Because of the loss of soluble carbohydrates these structural constituents at first rose rapidly in percentage but after several weeks leveled off. Where carbohydrate restorage took place later in the stubble of two series, namely the 50–60° and the 60–70°, cellulose dropped slightly and displayed an inverted U-curve. The roots made continual increases in both cellulose and pentosan and both plant parts made a steady increase in lignin, a normal aging process. The effect of temperature on these structural constituents was opposite to that noted on the soluble carbohydrates in that they were highest in the high temperature series. For brevity the effects of only two extreme conditions, the 50–60° and the 80–90°, on these substances in the stubble are illustrated in figure 4.

Total nitrogen of tops and stubble is illustrated in figure 5. A general downward trend in the total nitrogen of the new leaf growth as it ages is normal. In this experiment the series at the highest temperature showed the least decline. In the stubble the inclusion of new leaf area among the

older tissue might delay this downward trend but only in the highest temperature series was an increase noted. The total nitrogen of the roots is of

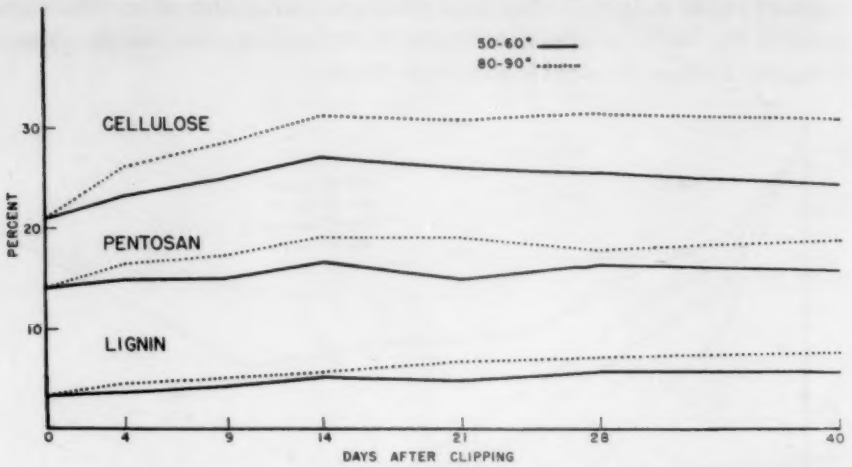

FIG. 4. Changes in the cellulose, pentosan, and lignin contents of the stubble of perennial ryegrass at two different temperature conditions (one replication only.)

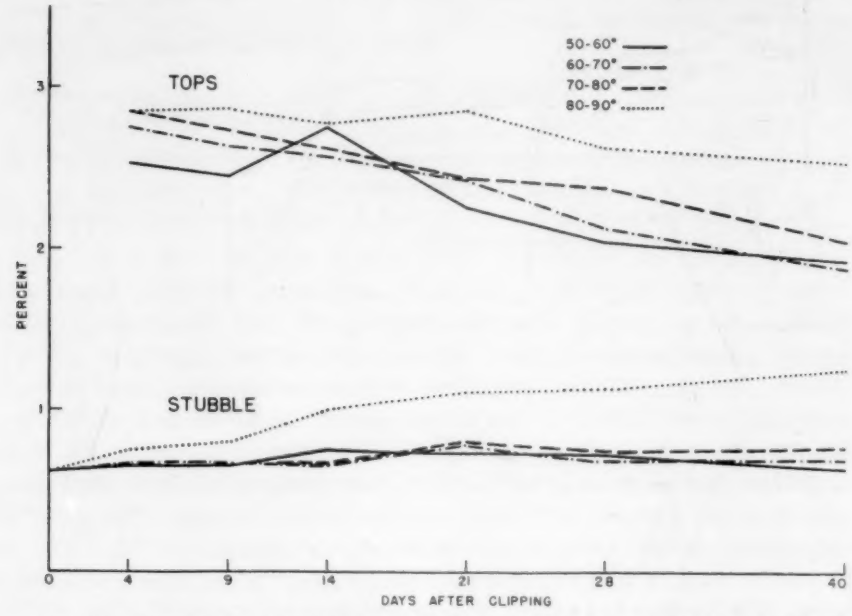

FIG. 5. Changes in the total nitrogen content of the tops anl stubble of perennial ryegrass at four different temperature conditions.

the same order as that of the stubble and gives much the same picture. The highest temperature series, 80–90°, was slightly superior to the other series in total nitrogen at the last three sampling dates. Since this series showed

the greatest loss in carbohydrates, its higher protein content may be apparent only.

That high temperature has some influence on the nitrogen metabolism is obvious when the soluble as well as total nitrogen is considered. Some data are illustrated in figure 6 in which the soluble nitrogen is expressed in

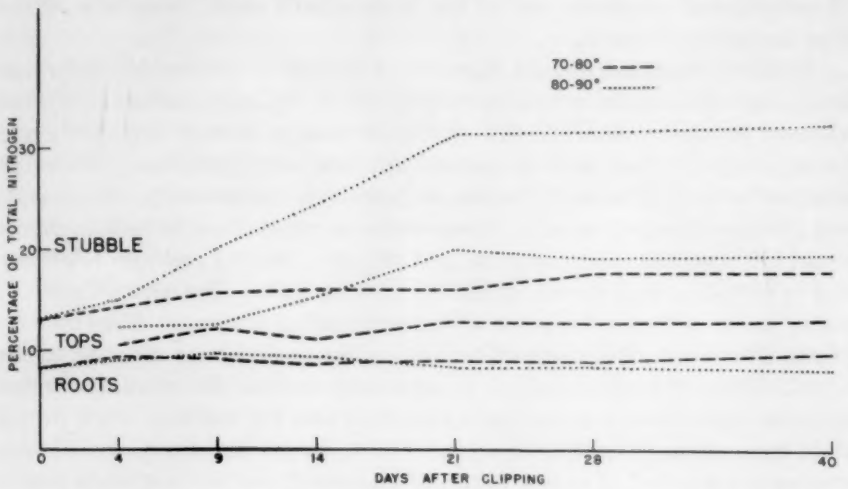

FIG. 6. Changes in soluble nitrogen, expressed as percentage of total nitrogen, in stubble, tops, and roots of perennial ryegrass at two different temperature conditions.

percentage of total nitrogen. The proportions of soluble nitrogen were distinctly higher in the stubble and tops in the 80–90° series than in the 70–80° and the latter was not greatly different from the other two low temperature series not shown in figure 6. The temperature effect on total nitrogen was not limited to an increase in soluble nitrogen; insoluble nitrogen was also higher in both the stubble and roots of the 80–90° series.

Total ash, phosphorus and calcium were also determined on the stubble and roots of the first replication. With the exception of a slightly higher calcium content of the stubble and roots of the highest temperature series at the later sampling dates no differences in these constituents were noted.

Discussion

These experiments demonstrate that high temperatures play a role in the productivity and survival of grasses. The range of temperatures at which the controlled chambers operated included the optimum temperature for the growth of new leaf tissue since its maximum yield occurred in one of the intermediate series, the 60–70°, and the maximum formation of carbohydrates also occurred within the range. At a temperature lower than the optimum for growth, synthesized carbohydrates were stored, but at temperatures higher than the optimum, they were rapidly dissipated without

having served as reserves in the sense that they contributed to the formation of new tissue. Whatever may be the effect of temperature on the rate of production of carbohydrates by photosynthesis, a rise in temperature will increase their loss by respiration. An excessive rate of respiration brought about by continued high temperature should lead to the exhaustion of carbohydrate reserves, and to the death of the plant, especially of one that has been defoliated.

Defoliation caused a rapid digestion of protein in the stubble and roots. In the highest temperature series, namely the 80–90°, the proportion of total nitrogen soluble in alcohol continued to increase at a more rapid rate and for a longer period than in plants at lower temperatures. While no analyses were made of this fraction, it contained, undoubtedly, amino acids and perhaps nitrogenous salts. Respiration is not confined to carbohydrates alone, but also affects amino acids and proteins, leaving residues relatively rich in nitrogen, such as amides and ammonium salts. The accumulation of one or more of these substances is characteristic of plants at high temperatures. The death of a plant at high temperature has been ascribed to the accumulation of ammonia (**1**). In these experiments the roots were more seriously injured by high temperatures than was the stubble. In a period of 28 days they changed color from white to brown and their sucrose and fructosan contents fell to near zero. It appears therefore that when plants are placed under adverse temperature conditions the roots are the first to die, an observation reported by others (**4, 11**). If the death of the roots is brought about by the exhaustion of carbohydrates, by the increase in ammonium salts or similar nitrogenous compounds and by an adverse effect of these compounds on protoplasm, the tops cannot survive long. The lower moisture content of the tops of the high temperature series may be a symptom of the failing capacity of the roots to take up water.

These experiments also suggest that temperature is one factor in the failure of perennial ryegrass to be adapted to the climate of much of the United States. The reduction of reserve carbohydrates with rising temperature agrees with the observation of BROWN (**3**) who found that nitrogen-free extract decreased with rising temperature in those species which normally grow in cool climates. In Bermuda grass he observed that nitrogen-free extract increased with rising temperature up to 100° F, the highest temperature he worked with. There is undoubtedly some relationship between carbohydrate accumulation and stability, and the ability of a plant to withstand high temperatures.

Plants at high temperatures behaved in some respects like plants that had been placed in darkness in a previous study (**12**), since both environmental conditions led to a rapid dissipation of carbohydrate reserves and to the digestion of protein. However, in one respect the nitrogenous metabolism was different. The plants in darkness accumulated a high concentration of nitrates which disappeared later, when the plants were

dying. No nitrates were found at any time in plants in these temperature studies though it must be noted that these plants grew in soil while those placed in darkness were in gravel cultures, constantly supplied with nutrients.

The effect of high temperature upon uncut and undisturbed plants cannot be determined from these studies but it appears from other work (**6**) that plants with high reserves, and incidentally plants not recently defoliated, are better able to withstand high temperatures than those recently cut. Defoliation alone (mowing or grazing) stimulates metabolic activities such as growth, nutrient absorption, translocation and respiration. The additional stimulus of high temperature at the same time may lead to unfavorable results by speeding up those processes, particularly respiration, and this may lead to an exhaustion of reserves.

Summary

Perennial ryegrass plants were clipped at a height of 1.5 inches above the surface of the soil and allowed to recover under controlled environmental conditions with four temperature variables, namely at 50–60, 60–70, 70–80, and 80–90° F, with daytime temperatures 10° above those of the night. Dry weight yields were taken and chemical analyses were made of the stubble, roots, and new top growth of plants under each of the four temperature conditions at 3, 9, 14, 21, 28 and 40 days after the beginning of the treatment. New top growth was most rapid at 60–70°, and least at 80–90°. In all four temperature series both roots and stubble decreased in total dry weight throughout the 40-day period following clipping. The losses were most rapid and extensive at the higher temperatures. The roots and stubble underwent rapid losses in sucrose and fructosan during the early part of the experiment but these losses were partly replaced later under the low temperature conditions. Under the highest temperature these losses were not replaced but continued, especially in the roots, almost to the point of exhaustion and in some cases to the death of the roots. Protein metabolism was characterized by an increase in the proportion of soluble nitrogen immediately after the leaves were clipped, and at the highest temperature this proportion continued to increase for a long period in the tops and stubble. Other constituents, lignin, cellulose, hemicellulose and ash, were not particularly affected by leaf removal or temperature except that their percentages were increased where soluble carbohydrates had been withdrawn.

In this experiment a high temperature adversely affected ryegrass by rapid dissipation of reserve carbohydrates, slowing down the production of new leaf growth, and in general inhibiting recovery from the effects of defoliation. At the end of a 40-day exposure to a temperature range of 80–90° F, the plants were stunted, the leaves were spindly and dark green, and the roots were discolored and near death. All parts were relatively

high in total nitrogen, and especially in soluble nitrogen, and were approaching exhaustion of soluble carbohydrates.

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EXPERIMENTS ON SUCROSE FORMATION BY POTATO TUBERS AS INFLUENCED BY TEMPERATURE[1]

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(WITH SEVEN FIGURES)

Received December 29, 1948

Introduction

It has been known that plants and plant parts maintained at low temperature tend to convert stored starch to sucrose. Thus MÜLLER-THURGAU (**15**) early found an accumulation of sugars and a corresponding loss of starch in potatoes kept at temperatures between 0° and 6° C. This work was later repeated and confirmed by WOLFF (**20**). Higher storage temperatures on the other hand result in an accumulation of reserve carbohydrates in the form of starch. Although the sweetening of potatoes, sweet potatoes and other tissues during low temperature storage has then been known for some time, the mechanisms responsible for the starch-sucrose conversion have remained obscure, due mainly to the fact that enzymes of the carbohydrate metabolism have been but little studied in higher plants. In animal tissues on the other hand a great deal is known concerning these matters. CORI and CORI (**2**) isolated from frog muscle glucose-l-PO_4 or Cori ester as the initial product of phosphorolysis of glycogen. Later they obtained the same ester from rabbit muscle (**3**) and showed that the presence of the enzyme phosphorylase, is responsible for the reaction. HANES (**8**) has shown that a similar but not identical phosphorylase is present in pea seeds and in potato tubers (**9**) and that this enzyme attacks starch with the production of glucose-l-phosphate. This ester may indeed be prepared biologically from starch with the aid of potato phosphorylase (HANES **8**, MCCREADY and HASSID **14**). The enzyme has also been found in yeast (**12**), and many other plant and animal tissues.

Although many plant tissues form sucrose on cold storage, the mechanisms involved are unknown. The enzymes of carbohydrate transformation in the potato tuber have been but little investigated, and no investigation appears to have been made on the relation of amount and activity of enzymes of the carbohydrate metabolism in relation to temperature. This paper will consider the carbohydrate metabolism of the potato both as to enzyme mechanisms and as related to temperature.

[1] Report of work supported in part by the Herman Frasch Foundation for Agricultural Chemistry.

Methods

Storage

Potatoes of the Russet variety were obtained from the market and were then randomly divided into groups. Each group was stored in the dark at controlled temperature ($\pm 1°$ C).

Sugar analysis

Samples of potato tissue were rapidly dried in a forced draft oven at 70° C. The dry samples were ground in a Wiley mill to 20 mesh and aliquots were then weighed out and extracted with 80 per cent. ethanol according to Hassid (**10**). After the extracts were cleared with lead acetate, aliquots were used for determinations of reducing sugars by the ceric sulphate method. Sucrose was determined as the increase in reducing power after hydrolysis with invertase. Non-sugar reducing substances were determined as the reducing power in aliquots of extracts which had been previously fermented with yeast. Fructose gives a characteristic color reaction with alcoholic resorcinol in strong acid solution (**16**) and was determined colorimetrically in a Klett-Summerson photoelectric colorimeter. Starch was analyzed in the residue of the alcohol extraction by hydrolysis either with amylase or with acid and titrated as glucose. In reaction mixtures, starch synthesis by phosphorylase was followed colorimetrically as follows: 1 ml. aliquots were removed from the digest to 1 ml. of 16 per cent. trichloroacetic acid in order to arrest the enzymatic activity. The samples were filtered and the precipitate washed several times. One-half ml. of iodine-potassium iodide solution was added and the sample was made up to 25 ml. final volume. The color developed was then measured in a Klett-Summerson colorimeter and compared with a calibration curve made for potato starch. This calibration is not an absolute one, and can be used only for the purpose of comparisons within an experiment.

Phosphorylated intermediates

A portion of dried tuber sample was extracted with successive portions of 5 per cent. trichloroacetic acid in the cold by a slight modification of the method described by Umbreit, Burris and Stauffer (**18**) until the acid extract gave a negative test for phosphorus. The phosphorylated intermediates in the combined extracts were determined by characteristic and specific reactions detailed in (**18**).

Phosphorus determinations

Inorganic phosphate and 7-minute-hydrolyzable-phosphate were determined by the colorimetric method of Fiske and Subbarow (**6**). One ml. samples for phosphate determination were removed from the digests to 16 per cent. trichloroacetic acid. After filtration 1 ml. of 2N sulphuric acid was added, followed by 1 ml. of 8.3 per cent. ammonium molybdate

solution and finally by 1 ml. of the reducing agent, 1-amino, 2-naphtol, 4-sulphonic acid. In the case of the samples for total phosphate the Cori ester was first hydrolyzed by placing the sample in boiling water for seven minutes after the addition of the sulphuric acid. After cooling the other reagents were added. In all cases the readings were made at exactly 20 minutes of color development.

The phosphate methods of KING (**13**) and ALLEN (**1**) were also used but showed no advantages over that outlined above.

Chemical changes in potatoes at different temperatures

The temperature at which potato tubers are stored has a profound effect not only on their content of sucrose but also on their content of other sugars and sugar derivatives, as is shown by the following experiment. Tubers of the variety Russet were stored at temperatures of 0°, 9°, 16° and 25° C. An initial sample was taken as the potatoes came from common storage and further samples were taken after two weeks of controlled temperature storage. These samples were analyzed by the procedures described above. The results of this experiment which are given in Table I, lead to the following conclusions:

TABLE I

CARBOHYDRATE COMPOUNDS AND DERIVATIVES IN POTATO TUBERS AS INFLUENCED BY TEMPERATURE OF STORAGE

VALUES GIVEN IN PER CENT. OF DRY WEIGHT

SUBSTANCE ANALYZED	ZERO TIME (MARKET)	AFTER TWO WEEKS OF STORAGE			
		0°	9°	16°	25°
Starch	67.00	61.00	65.00	63.00	64.00
Glucose	.62	.79	.73	.49	.56
Fructose	.17	1.50	.34	.22	.15
Sucrose	1.07	6.65	1.25	.75	.84
Non-sugar red. substances	.00	.60	.09	.19	.09
Glucose-1-phosphate	.00	.17	.04	.00	.00
Glucose-6-phosphate	3.50	.70	.66	4.20	4.50
Fructose-6-phosphate	.17	2.50	1.05	.25	.35
Fructose-1,6-diphosphate	.00	.00	.00	.00	.00
Triose phosphates	.37	.94	.11	.57	.26

1. Starch concentration decreased in all conditions, the reduction being most marked at 0° C.
2. Glucose concentration increased slightly during storage at 0° and 9° but decreased a little at the higher temperatures.
3. A remarkable change occurred with respect to fructose which increased in concentration nearly ninefold at 0° and twofold at 9° C but remained approximately constant in concentration at the two higher temperatures.
4. Striking changes occurred in concentrations of glucose-6-phosphate and

fructose-6-phosphate. The first compound was found in greater quantities at 16° and 25° C than at temperatures of 0° and 9° C. The reverse was true for fructose-6-phosphate.

5. Glucose-1-phosphate was found in detectable concentration only in tubers stored at 0° or at 9°.

These results indicate that investigation of the problem of sugar formation in potato should include study of the metabolism of the phosphorylated sugars.

Phosphorylase

The phosphorylase content of potatoes from various storage temperatures was first studied. The standard procedure developed for obtaining

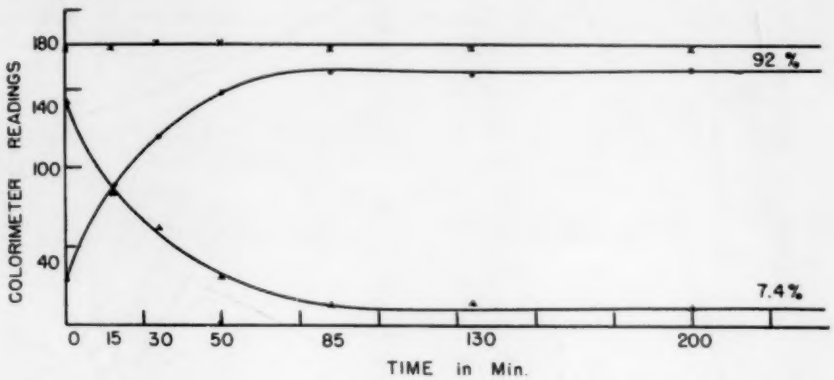

FIG. 1. Activity of potato phosphorylase in synthesis of starch as measured by the changes in phosphates. Digest containing 5 ml. of 5× concentrated enzyme, 3 ml. of maleate buffer pH 5.5, 30 mgs. of Cori ester, and 2 mgs. of starch. Total volume of reaction mixture 20 ml. Incubated at 25° C.

Inorganic phosphate = ●, Cori ester = ▲, total phosphate = ×.

the enzyme from potato consisted of a modification of that used by GREEN and STUMPF (**7**), and by HANES (**9**), both of whom make use of a fractional precipitation of the proteins present in potato juice with ammonium sulphate. A comprehensive study of the method showed that potato proteins should not remain in contact with ammonium sulphate during precipitation any longer than is absolutely essential, since long contact causes losses in enzyme activity. A layer of toluene over the crude juice prevents oxidation. Low temperatures are recommended during extraction, and keeping the enzyme preparation wet or otherwise in solution is also part of the technique.

ACTIVITY

Standard digests consisting of definite quantities of enzyme, substrate and primary substances (starch) were used in the course of the experiments for determination of relative phosphorylase activity. The standard digest consisted of enzyme peparation (semi-purified) 5 ml., Cori ester 30

mgs. (3 mg./ml. solution), starch 2 mgs., and 3 ml. 0.1 M maleate buffer pH 5.5, (total volume 20 cc.) The incubation temperature was 25° C unless otherwise stated. Phosphorylase activity may be followed by determination of the inorganic phosphate liberated during conversion of Cori ester to starch. The results of a typical experiment of this kind are given in figure 1. In a similar manner the amount of starch synthesis can be followed colorimetrically and figure 2 shows the colorimeter readings of the iodine-starch complex plotted against time for the same reaction mixture as shown in figure 1. The color developed rises to a maxi-

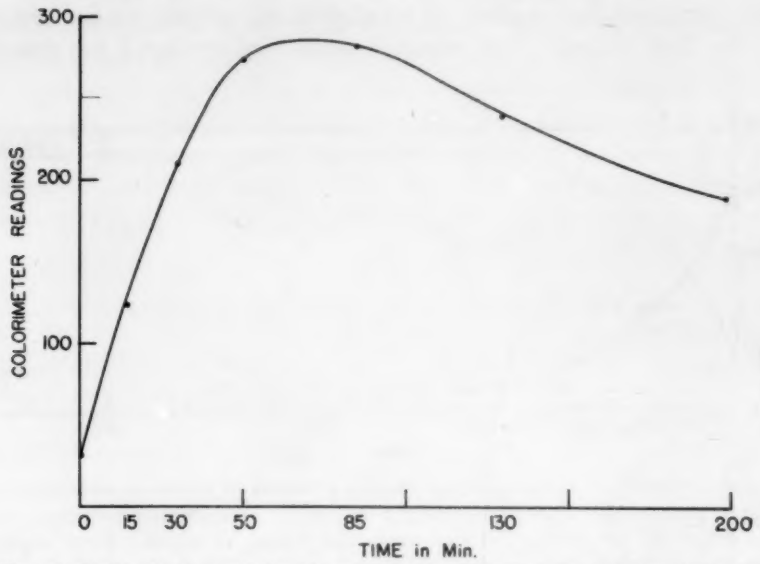

FIG. 2. Determination of phosphorylase activity as measured by synthesis of starch from glucose-1-phosphate. Conditions identical with those of figure 1.

Ordinates represent the colorimeter readings of the iodine-starch complex.

mum and then decreases after equilibrium has been attained as judged by the attainment of constant phosphate concentration in figure 1. During the interval in which these readings were taken the color of the starch-iodine complex solution changed from a deep blue to a violet blue, followed by a violet reddish color, suggesting that the starch has undergone some structural change resulting in decreased absorption of light by the starch-iodine complex. It is known that starch consists of two fractions which are designated as amylose and amylopectin. The former is easily soluble in H_2O and forms a slightly viscous solution, retrogrades from solution, and consists of straight unbranched chains of glucose molecules joined in 1–4 linkages. Amylose produces deep blue color with iodine and is the form of starch produced initially by the action of phosphorylase on Cori ester. The amylopectin fraction is less soluble, gives a highly viscous, opalescent solution in H_2O, produces a violet-red or reddish color

with iodine and is formed of chains of glucose residues linked through 1–4 linkages, but with frequent branches at position 6 of the gluco-pyranose residues. In view of these properties of the starch fractions, the change in color of the starch iodine complex after attainment of phosphate equilibrium suggests and gives some support to the theory that the enzyme preparation contains the "Q" factor of HAWORTH and co-workers (**11**) which enables phosphorylase to form 1,6-glycosidic linkages. This must involve conversion of 1–4 linkages since no net phosphate exchange is involved.

ACTIVITY OF VARIOUS CONCENTRATIONS OF PHOSPHORYLASE

A highly concentrated enzyme was prepared and then diluted to give a range of concentrations. These concentrations were referred to the

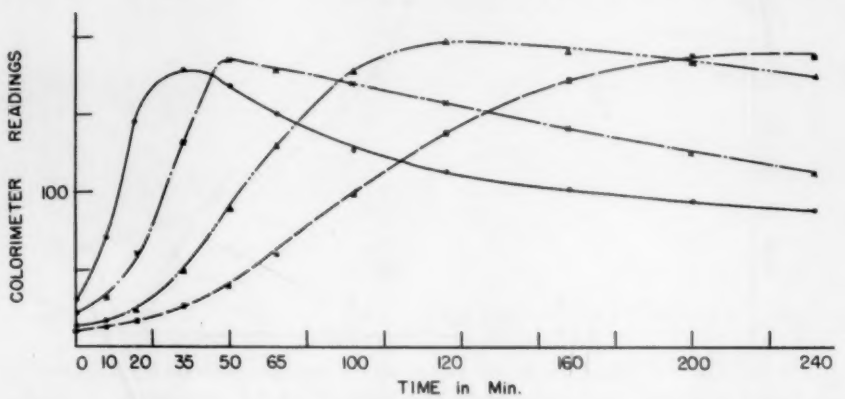

FIG. 3. Rate of synthesis of starch by various concentrations of phosphorylase. The reaction was followed by starch synthesis and the amount of starch measured colorimetrically. Digest consisted of the same amounts and components as for figure 1, with the exception of varying enzyme concentrations.

Enzyme concentrations: 20X ———— ● (20X concentrated over pressed juice)
10X ——— · ——— × 5X ——— - - ——— ▲ 2.5X —— —— —— ■

original volume of potato juice used in the fractionation. Thus a 20x concentrated enzyme is one which, after purification, was taken up in 1/20 of the original juice volume. Various concentrations of enzyme were then incubated with Cori ester as described above and reaction rates followed by determination of the starch formed. Results of such an experiment are represented graphically in figure 3 in which time of incubation is plotted against the colorimeter readings of the starch-iodine complex. From the initial slopes of the curves of figure 3 it can be deduced that the rate of starch synthesis by potato phosphorylase is proportional to the enzyme concentration within the range of concentrations studied. The final equilibria attained and the total amounts of starch synthesized from equal amounts of Cori ester were the same for all enzyme concentrations.

Effect of pH on phosphorylase activity and equilibrium

The pH in the phosphorylase reaction mixtures was adjusted by means of maleate (**17**) and borate buffers, other factors being kept constant. The results of a typical experiment over the range pH 4.5 to 7.5 are summarized graphically in figure 4, where the slopes in the linear portions of

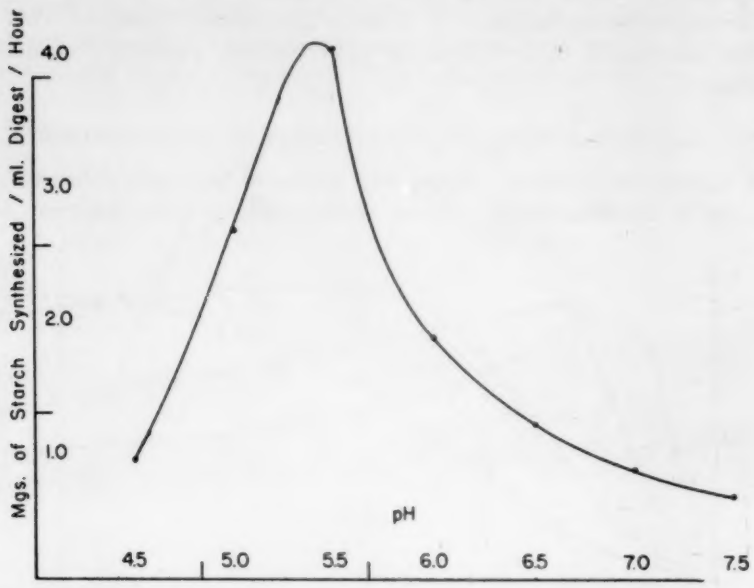

Fig. 4. Effect of pH on the initial rate of starch synthesis reaction by phosphorylase. The digest contained same components and amounts as for experiment of figure 1 except for the pH of the buffer which varied as indicated in the abscissae.

the starch formation-time curves are plotted as a function of pH. As expected, hydrogen ion concentration markedly affected the rate of phosphorylase action, maximum rate occurring at approximately pH 5.5. Above or below this value there is a decrease in rate which is more noticeable towards the less acid side.

TABLE II

Effect of pH on the phosphorylase equilibrium

Digests contained 30 mgs. of Cori ester, 5 ml. of enzyme preparation, 3 ml. of buffer and 2 mgs. of starch. The incubation temperature was 25° C.

pH of digest	4.5	5.0	5.5	6.0	6.5	7.0	7.5
mgs. of starch per ml. of digest at equilibrium	1.04	.99	.98	.95	.90	.85	.78

The pH not only affected the rate of reaction but also the position of the final equilibrium. Under more acid conditions a larger portion of the Cori ester in the digest is converted to starch and inorganic phosphate as can be seen below in table II.

The phosphorylase equilibrium is determined by the concentration of the divalent phosphate and glucose-1-phosphate anions (**4, 9**). Phosphate and glucose-1-phosphate have different dissociation constants. The pH of the medium therefore influences the ratio of total phosphate to total glucose-1-phosphate present in the equilibrium mixture.

Effect of substrate concentration

Several experiments were carried out using 15, 30 and 60 mgs. of Cori ester in digests containing the same enzyme preparation and standard amounts of buffer and priming substance. Figure 5 shows that in such an

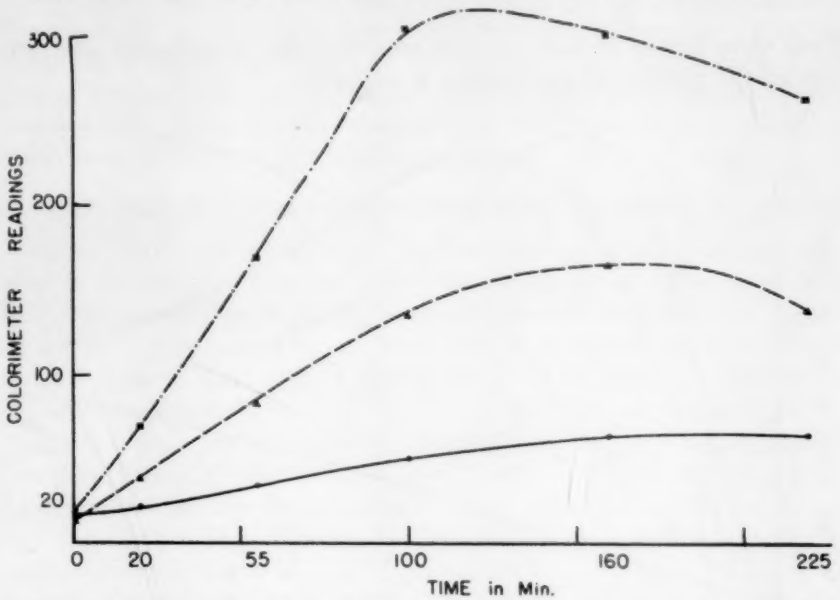

Fig. 5. Effect of substrate concentration on phosphorylase activity as followed by starch synthesis. The digest contained the same components as described for previous experiment 1. Amount of Cori ester was varied in the reaction mixtures as indicated below.

Amount of Cori ester in digest:
● ——— 15 mgs.
▲ — — — — 30 mgs.
■ — . — . 60 mgs.

experiment, in which all other conditions were held constant, the rate of starch synthesis was proportional to the amount of Cori ester added. Similarly the rate of inorganic phosphate liberation was proportioned to the substrate concentration. The position of the final equilibrium was not however appreciably influenced. Calculating the equilibrium for the three concentrations of substrate, table III was obtained.

Effect of temperature

Experiments were carried out with all factors constant except temperature of the digest. The temperatures used were 0°, 10°, 20°, and 30° ± 0.5° C. Rate of starch synthesis was used as the measure of enzyme activity. The results of figure 6 show that the rate of reaction in-

TABLE III

EQUILIBRIUM ATTAINED IN EXPERIMENT OF FIGURE 5 IN TERMS OF PHOSPHATE

DIGESTS CONTAINED THE SAME CONCENTRATION OF ENZYME BUFFER AND PRIMING SUBSTANCE

DIGEST NO.	AMOUNT OF CORI ESTER ADDED TO DIGEST IN MGS.	INORGANIC PHOSPHATE % OF TOTAL	ORGANIC PHOSPHATE % OF TOTAL
1	15	11%	85%
2	30	9%	88%
3	60	9%	90%

From these figures we may conclude that the effect of substrate concentration on the position of equilibrium is negligible.

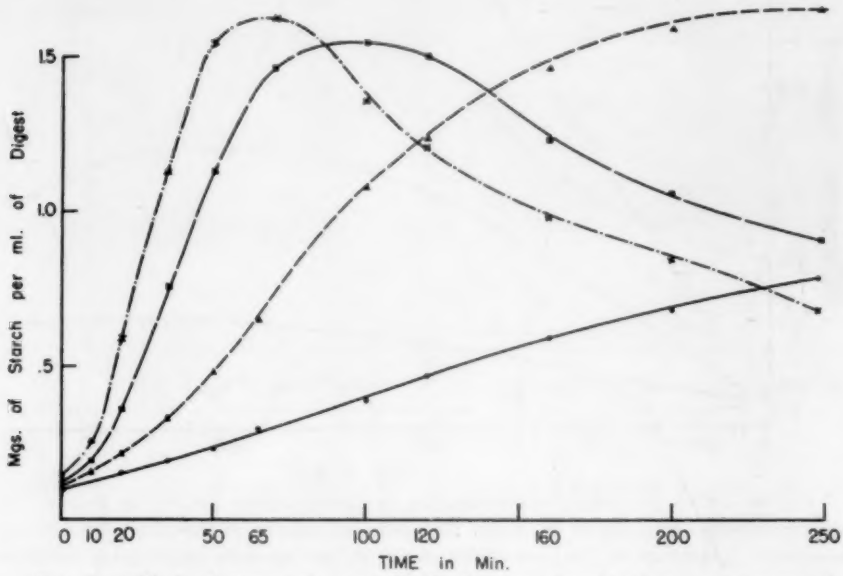

FIG. 6. Effect of temperature of incubation on phosphorylase activity reaction mixtures prepared in the same way as for experiments of figure 1.

The temperatures of incubation were in °C

——— ● 0° — — — — ▲ 10° ——— ——— ■ 20° x —— . —— × 30°

creases with increasing temperature up to 30° C. The final equilibrium position was not affected by temperature since the final amount of starch synthesized when equilibrium was attained was the same at all experimental temperatures used, as must be expected for a process associated with an insignificant production of heat. The rate of reaction was, however, very much influenced by temperature. Calculating the temperature coefficient Q_{10} for the rates plotted in figure 6 at three different points we obtain the following, where

$$Q_{10} = \frac{\text{velocity at } T^\circ + 10^\circ}{\text{velocity at } T^\circ}$$

TABLE IV

EFFECT OF TEMPERATURE ON RATE OF REACTION OF PHOSPHORYLASE

TIME IN MINUTES	Q_{10} BETWEEN			
	0–10°	10–20°	20–30°	30–40°
20	1.41	1.77	1.53	.94
35	2.00	2.17	1.50	.84
50	2.30	2.13	1.32	.74

The values for Q_{10} lie in the range 1.3–2.3 except at the highest temperatures where the Q_{10} is smaller than 1 and decreases consistently with time, suggesting a rapid destruction of the enzyme. It has been shown by others (7) that 61 per cent. of the activity of phosphorylase is lost by heating at 58° for three minutes while heating for the same time at 68° C results in a 97 per cent. loss of activity.

EFFECT OF VARIOUS TEMPERATURES OF STORAGE ON PHOSPHORYLASE ACTIVITY

Experiments were carried out to determine the effect of storage temperature on the phosphorylase activity of potato tubers. The purpose of these experiments was to determine quantitatively the changes in enzyme content and enzyme activity as a response to temperature and time of storage. This is particularly pertinent; it is the starch which is the source of the sugars, formed in response to low temperature storage.

Potatoes from the market were randomized into four lots of approximately 10 lbs. each. The lots were stored at 0°, 10°, 20° and 30° C. Initial determinations of phosphorylase content and activity were made, and after varying periods of storage further samples were taken for study of enzyme content. Comparisons of phosphorylase activity were based on the rate of starch formation at 25° C in reaction mixtures prepared with standard enzyme preparations. The results of a typical storage experiment are given in table V, in which each value represents the average of three enzyme extracts for each batch of potatoes.

TABLE V

TEMPERATURE OF PREVIOUS STORAGE HAS NO INFLUENCE ON THE AMOUNT OF PHOSPHORYLASE CONTAINED IN POTATO TUBERS. FOR DETAILS OF REACTION MIXTURE SEE TEXT

TEMPERATURE OF STORAGE IN °C	TIME OF STORAGE IN WEEKS	TEMPERATURE OF REACTION IN °C	RATE OF STARCH FORMATION IN MGS./ML. DIGEST/HR.	MGS. OF STARCH/ML. OF DIGEST AT EQUILIBRIUM
Market	0	25°	2.0	1.5
0	3	"	2.1	1.3
10	3	"	1.6	1.2
20	3	"	2.0	1.4
30	3	"	2.0	1.5

The results show that the activity of phosphorylase as judged from the partially purified preparation remained approximately constant under all storage conditions. The equilibria were also reached at the same time and the final amount of starch formed was roughly the same in all cases. When the digests were incubated at the same temperature as that of storage the rates of reaction, as is to be expected, were a function of temperature of incubation as discussed above.

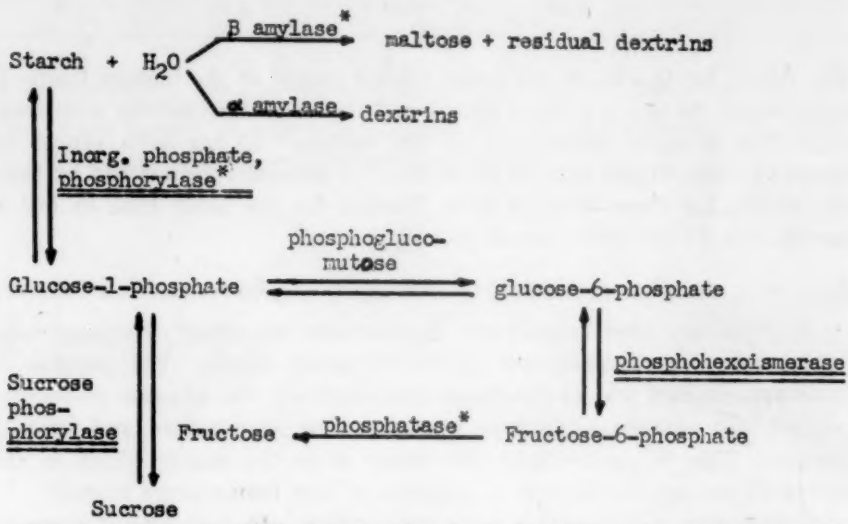

FIG. 7. Pathways of starch metabolism in plant tissues. All of the enzymatic mechanisms noted have been found in one or more species of plants and all but sucrose phosphorylase in higher plants. Those reactions marked * have been studied with isolated enzyme systems in present work. The others have been studied in tissue slices. Those reactions which are doubly underlined are those which appear to be influenced by temperature of storage of the potato tuber.

It can be concluded then that the activity of phosphorylase found in the potato tuber is not affected by temperature of storage. The starch-Cori ester equilibrium is likewise little influenced by temperature.

REGULATION OF PHOSPHORYLASE ACTIVITY

Table 1 shows that there is no appreciable amount of Cori ester in potatoes stored at high temperatures. Since inorganic phosphate and starch are present, a non-equilibrium condition obtains with regard to this system. Why then is starch not attacked by phosphorylase in potatoes stored in the warm as it is in potatoes stored in the cold? It appears that there may be a substance or substances in potatoes stored at 25° C which inhibit phosphorylase activity and that this factor is absent or present in lower concentration in potatoes stored at 0° C. Evidence to substantiate this idea is found in the following experiments.

Potatoes from 0° or from 25° storage were ground in a Waring blendor and extracted with boiling 80 per cent. ethanol. The alcohol was then dis-

tilled off, and the aqueous solution, which will be referred to as inhibitor, was filtered and made to volume, so that each ml. of inhibitor represented 1 gm. of fresh potato. The inhibitor preparations from tubers stored at 0° or at 25° C were added to reaction mixtures containing 5 ml. of 2x enriched phosphorylase, 2 mgs. starch, 20 mgs. of Cori ester, and maleate buffer of pH 6.5, the total volume amounting to 10 ml. One ml. samples were removed periodically and the amount of starch synthesized followed. The results of such an experiment are found in table VI.

TABLE VI

EFFECT OF 80 PER CENT. ALCOHOL EXTRACTS OF POTATO ON THE ACTIVITY OF POTATO PHOSPHORYLASE IN STARCH SYNTHESIS EXPERIMENT

DIGEST NO.	CONCENTRATION OF INHIBITOR	SOURCE OF INHIBITOR	INITIAL REACTION VELOCITY STARCH FORMED MG./HR./ML.
1	none	.....	1.12
2	Represents 0.5 gm./potato/ml.	Tubers Stored at 0°	1.36
3	Represents 0.5 gm./potato/ml.	Tubers Stored at 25°	0.26

The data indicate that the preparation from potatoes stored at 25° C inhibits the synthesis of starch from Cori ester by phosphorylase. The inhibitor seems to be absent from potatoes stored at 0° C, where polysaccharide synthesis took place at a rate approximating that in the controls. Similar results were obtained when the 80 per cent. ethanol extracts were studied with respect to their effects on the degradation of starch by phosphorylase. In the preparation of the enzyme from potato tubers, phosphorylase is apparently separated from inhibitor by the ammonium sulphate precipitation procedure. Thus even though as much phosphorylase is found in potatoes stored at high temperatures as in potatoes stored at low temperatures, still the activity of the enzyme *in vivo* may be decreased at high storage temperatures by the inhibitor.

Amylase

The role of amylase in starch hydrolysis in potatoes stored at various temperatures was investigated. Whole juice pressed from potatoes was used. The enzymes were then precipitated with half saturated ammonium sulphate, the precipitate taken up in water and dialized. Dialysis against distilled water inactivated phosphorylase, permitting a separation of amylase from phosphorylase. The enzyme found in potato juice is a beta-amylase which converts starch into maltose and dextrins. No attempts were made to study the bound amylase in tubers.

ACTIVITY

Activity was determined by a modification of WOHLGEMUTH'S (**19**) method, in which the disappearance of color of the iodine-starch com-

plex is followed as a function of time. Acetate buffer pH 5.0 was used in all digests and the reaction mixtures were incubated at 25°C. In the following experiment the reaction mixtures contained 5 ml. of 2x concentrated enzyme preparation, 3 ml. of acetate buffer, and 2.5 mgs. of starch/ml. digest. The total volume amounted to 20 ml. One ml. aliquots were removed periodically, I_2-KI reagent added and the residual starch determined colorimetrically. The results of this experiment are presented in table VII. Potatoes from 30° storage showed a higher amylase activity

TABLE VII

EFFECT OF TEMPERATURE OF STORAGE ON AMYLASE CONTENT OF POTATO TUBERS

THE FIGURES REPRESENT AVERAGE VALUES OF FOUR EXPERIMENTS

TIME OF STORAGE FIVE WEEKS	TEMPERATURE OF STORAGE			
	0°	10°	20°	30°
Mgs. of starch hydrolyzed in 200 200 min./ml. of digest	0.37	0.29	0.27	0.43
% of starch hydrolyzed in 200 min.	14.8	11.6	10.8	17.2

than potatoes from any other condition. Digests containing amylase and incubated at 0° showed that this temperature essentially suppressed amylase activity. These results taken together suggest that the amylase present in the potato cannot account for the starch hydrolysis in potatoes stored at 0°, and hence cannot be related to the normal sweetening of tubers stored at lower temperatures.

Phosphatase

Among the phosphatases known in plant tissues are those which attack hexose phosphates to yield the corresponding hexoses. Since it is conceivable that phosphatase might in some way be involved in the starch-sucrose transformation in the tuber, the presence and activity of this enzyme in potatoes from various storage temperatures was studied.

ACTIVITY

Whole juice and ammonium sulphate fractionated juice were used as the enzyme source. The precipitate from 50 per cent. saturation of whole juice yielded the most active fraction. The enzyme preparation liberated inorganic phosphate from fructose-6-phosphate and was also able to attack fructose-1,6-diphosphate. The pH optimum of this potato phosphatase is in the neighborhood of pH 8.0 and the enzyme is inhibited by potassium fluoride (50 per cent. inhibition with 0.02 mg. KF per ml.) For the following experiment, 5.0 ml. aliquots of 1× concentrated enzyme were taken from potatoes which had been stored at 0° or at 25°C for three weeks. The reaction mixtures also contained 10 ml. of a saturated solution of the

calcium salt of fructose-6-phosphate, 2 ml. of 0.1M $MgCl_2$ and 3 ml. of borate buffer pH 8.0. The results are given in table VIII. The data show

TABLE VIII

EFFECT OF TEMPERATURE OF STORAGE ON POTATO PHOSPHATASE

INCUBATION TEMPERATURE OF REACTION MIXTURES 25° C.		
TEMPERATURE OF STORAGE	TIME OF STORAGE	MGS. OF INORGANIC PHOSPHATE LIBERATED IN 250 MIN./ML. OF DIGEST
Market	0	0.11
0	3 weeks	0.14
25	"	0.14

that the differences in activity of potato phosphatase from potatoes stored at 0° and 25° were negligible. Phosphatase activity was, further, completely suppressed at 0° C.

Experiments *in vivo*

The experiments reported above have given a clue as to the nature of the temperature-controlled reaction which regulates starch breakdown, e.g. the production of a phosphorylase inhibitor at high temperature. None of the results obtained with isolated enzyme systems have however given any information as to how sucrose is formed in the potato tuber. *In vivo* experiments were therefore carried out, using discs of living tissue cut from potato tubers which had previously been stored at various temperatures.

METHODS

Potatoes were stored for varying periods of time at 0°, 9°, 16°and 25°C. Cylinders then were cut out with a cork-borer and these sliced into discs 1 to 2 mm. thick. The discs were washed in running tap water for 20 minutes and rinsed in distilled water. They were then vacuum infiltrated with the desired substrates. Controls were run in each experiment by infiltrating the discs with distilled water under exactly the same conditions. After infiltration the discs were transferred to Petri dishes containing a sheet of filter paper moistened with the same infiltration solution to keep the atmosphere around the discs moist. The discs were incubated at the desired temperature and after 24 hours were washed and dried at 70° C, ground and subjected to analysis as described earlier.

SYNTHESIS OF SUCROSE BY POTATO DISCS

Sucrose is synthesized by potato discs which are infiltrated with aqueous solutions of glucose and/or fructose, a result which strongly suggests that the two simple sugars are used as intermediates in sucrose synthesis.

Table IX summarizes the results from a typical experiment in which discs were taken from potatoes stored either at 0° or at 25°C and which were then infiltrated with either glucose or fructose. The following conclusions can be drawn from this and similar experiments:

1. In discs from tubers which had been stored at 0°, sucrose synthesis in general occurred at nearly the same rate when glucose was supplied as when the same concentration of fructose was infiltrated.

2. Discs from tubers stored at 25°C formed more sucrose when given fructose than when given glucose, which is in accord with the data of table I, which shows very low fructose concentrations in such tissue.

TABLE IX

SYNTHESIS OF SUCROSE BY POTATO DISCS AFTER INFILTRATION WITH GLUCOSE OR FRUCTOSE. ALL TISSUES INCUBATED 24 HOURS

SUBSTRATE INFILTRATED	INCUBATION TEMPERATURE °C	INCREASE IN SUCROSE CONTENT DURING INCUBATION: % OF DRY WT.	
		STORAGE TEMPERATURE	
		0°	25°
Water	0	1.80	0.52
	25	−0.50	0.46
1% glucose	0	3.00	0.48
	25	0.65	0.60
1% fructose	0	2.85	0.96
	.....	.....	1.90
3% glucose	0	4.00	0.76
	25	2.15	1.85
3% fructose	0	3.65	1.26
	25	2.15	1.97

3. In spite of the fact that discs from tubers stored at 0° contained more sucrose than discs from tubers stored at 25°, the former were more effective in sucrose formation irrespective of temperature of incubation.

4. In discs from tubers stored at 0°, incubation at 0° induced a greater increase of sucrose than incubation at 25°C, indicating that low temperature favors the process of sucrose synthesis itself as well as the process of starch breakdown.

In summary, potato sections synthesize sucrose rapidly when they are provided with hexose. The amount of sucrose synthesized is greater in sections taken from potatoes stored at low temperatures, suggesting that low temperature storage may increase the amount of the sucrose synthesizing enzyme system, whatever it may be. Sucrose synthesis, unlike the other reactions studied above, proceeds even faster in sections incubated at 0° than in sections incubated at 25°C. The mechanism which brings about this unusual reaction to temperature is obscure.

Interconversion of glucose and fructose

It was not possible to demonstrate phosphohexoisomerase *in vitro* in potato preparations. Glucose and fructose were, however, interconverted in potato discs, as was shown by infiltrating discs with glucose or fructose and then analysing for these two hexoses after an incubation period.

The results of an experiment designed to study this interconversion are given in table X, which gives the increases of glucose and fructose con-

TABLE X

Interconversion of glucose and fructose in potato discs after infiltration. Incubated 24 hours

Infiltrated with	Incubation temperature °C	Increases in hexose above level in H_2O infiltrated controls % of dry weight			
		Tubers stored at 0°		Tubers stored at 25°	
		Glucose	Fructose	Glucose	Fructose
10% glucose	0	2.04	1.15	0.40	0.64
	25	3.20	0.38	0.78	0.29
10% fructose	0	1.63	0.69	0.52	0.69
	25	2.80	0.34	0.00	0.18
3% glucose	0	2.65	0.80	0.52	0.75
	25	1.36	0.24	0.30	0.06
3% fructose	0	1.30	0.25	0.63	0.80
	25	1.79	0.25	−0.28	0.07

centrations during an incubation period of 24 hours. The data of this and similar experiments may be summarized as follows:

1. Discs from tubers stored at 0° appear to take up more glucose and more fructose than discs from tubers stored at 25°C.

2. Storage at 0° and incubation at 0° result in large interconversions of glucose and fructose.

3. Storage at 25° and incubation at 25° result in small interconversion of the two sugars.

4. Storage at 25° and incubation at 0° or *vice versa* give intermediate behavior.

In conclusion it can be said that the interconversion of glucose and fructose is promoted by low temperatures. This effect is in the same direction as that found for sucrose synthesis of which the interconversion reaction is undoubtedly a part.

Discussion

The known reactions relating starch, hexoses, and sucrose in the plant and in microorganisms are summarized in figure 7. Two methods for the breakdown of starch are well-established; namely, the hydrolytic breakdown under the influence of amylase and the phosphorolytic breakdown

under the influence of phosphorylase. The potato tuber possesses only weak amylase activity. It does on the other hand possess a powerful phosphorylase. The action of phosphorylase on starch results in the production of glucose-1-phosphate. The breakdown of starch in potatoes stored at low temperature evidently proceeds through the phosphorolytic system since (a) low temperatures conducive to *in vivo* starch breakdown in the potato essentially completely suppress amylase activity and (b) during active starch breakdown glucose-1-phosphate appears in the potato in detectable quantities, whereas it is absent from potatoes which are not undergoing starch breakdown. Potatoes stored at high temperature possess as much phosphorylase as potatoes stored at low temperature. They also possess inorganic phosphate and a pH favorable to phosphorylase activity. Why then does starch remain unattacked by phosphorylase in potatoes stored at high temperature?

The answer appears to lie in an inhibitor of phosphorylase activity which is found in potatoes stored at high temperatures and which is absent or present in lower concentrations in potatoes stored at low temperatures. Since, however, starch is presumably formed in the tuber even at temperatures in which starch breakdown does not occur during subsequent storage, it would appear that this inhibitor must be formed relatively late in the development of the tuber. In general much remains to be explained concerning the formation, degradation, and nature of the phosphorylase inhibitor.

The formation of sucrose from glucose-1-phosphate involves the production of fructose. The sole known method for the biological interconversion of glucose and fructose is through the enzyme phosphohexoisomerase, an enzyme which establishes an equilibrium between glucose-6- and fructose-6-phosphate. This enzyme could not be demonstrated in potato tubers during the present investigation, owing perhaps to inadequate preparative techniques. *In vivo* experiments of many workers have shown however that glucose and fructose are interconvertible and this has been shown again for the potato tissue. Lastly glucose and fructose once formed are combined in some way to sucrose. In the microorganism *Pseudomonas saccharophila* DOUDOROFF, KAPLAN, and HASSID (**5**) have shown that sucrose synthesis is achieved from glucose-1-phosphate and fructose in the presence of an enzyme, sucrose phosphorylase. The existence of this reaction and of this enzyme in potato tuber tissue could not be established during the course of the present work and the exact mode of sucrose formation is hence unknown. Sucrose synthesis was however readily achieved *in vivo* after infiltration with either glucose or fructose and in particular with tissue taken from tubers which had previously been stored at the low temperatures conducive to the sweetening reaction.

The regulations of starch degradation and sucrose synthesis by temperature in the potato tuber would appear to reside in at least three re-

actions, (a) regulation of the amount of phosphorylase inhibitor which is produced in high temperatures, (b) regulation of the activity of the glucose-fructose interconverting system which is increased in amount in low temperature storage, and (c) regulation of the activity of the sucrose synthesizing enzyme which is increased in amount in low temperature storage.

Summary

1. Analysis of potato tubers during storage at low and high temperatures shows that changes take place in the composition of the tubers, not only with regard to starch (which decreases in low temperatures), hexoses and sucrose (which increase at low temperatures), but also with regard to hexose phosphates. The balance between glucose-6 and fructose-6-phosphate in particular is greatly affected, the latter being favored at the lower storage temperatures.
2. Potatoes contain only low amylase activity and this enzyme is essentially inactive *in vitro* at 0°. This enzyme appears not to be a factor in starch degradation and sugar formation at low temperatures.
3. Phosphorylase is equally active in potatoes from all storage temperatures. At no temperature was equilibrium between Cori ester and starch attained *in vivo*. The fact that phosphorylase does not attack starch in potatoes stored at high temperatures seems to have its explanation in the fact that an inhibitor of phosphorylase is formed at high storage temperatures, but disappears at low storage temperatures.
4. Sucrose formation from hexose infiltrated *in vivo* is promoted at low temperatures. Whether this is due to an increase in amount of the enzyme system responsible for sucrose synthesis or to a decrease in a possible inhibitor cannot be stated.
5. Hexose interconverting enzymes are more active at low temperatures and in tubers stored at low temperatures than at high temperatues. This observation is correlated with the fact that tubers stored at low temperatures are high in fructose and fructose-6-phosphate whereas those stored at high temperatures are high in glucose and glucose-6-phosphate.

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EFFECTS OF VARIATIONS IN THE ROOT–LEAF RATIO ON TRANSPIRATION RATE

JOHNSON PARKER

(WITH THREE FIGURES)

Received November 15, 1948

When conditions are favorable for relatively rapid transpiration the rate of water loss is often determined by the rate of absorption. If soil moisture is not limiting this rate depends largely on the extent and efficiency of the absorbing surface, that is, the root system. Variation in the ratio of root surface might therefore be expected to affect the rate of transpiration. FOWELLS and KIRK (**2**) found that removal of part of the root system of pine seedlings reduced transpiration, and MARSHALL and MAKI (**5**) believed that transpiration of pine seedlings might be related to size of the root system. BIALOGLOWSKI (**1**) was able to demonstrate a quantitative relationship between root-leaf ratio and the water loss per unit of leaf surface with rooted lemon cuttings. In order to obtain further information on this relationship for various woody species, the following experiments were carried out.

Methods and results

The transpiration rates of four intact individuals of each of four species were measured in the greenhouse over a period of two weeks. The plants used were two-year-old seedlings of red pine (*Pinus resinosa* Ait.), loblolly pine (*P. taeda*, L.), eastern red cedar (*Juniperus virginiana*, L.) and two year old rooted cuttings of privet (*Ligustrum japonicum*, Thunb.). Plants were transplanted from the clay pots in which they had been grown to metal containers without disturbance to the soil mass or the roots. It had been assumed that considerable differences in ratio of root to leaf surface would exist in a mixed population of seedlings such as the ones used in this experiment. Actual measurements proved this assumption to be correct and the variation in root leaf ratios is shown in figure 1.

The tops of the containers were covered with two layers of oil cloth to prevent water loss from the soil and the containers were weighed each morning and evening, water lost being replaced each third day. After two weeks of measurements the root systems were washed free of soil and the dry weights of roots, stems, and leaves were obtained for each plant. Leaf surface was estimated on a photoelectric apparatus after removal from the stems (**3**). Because of different leaf shapes and volume-area relationships of different species, considerable difference in estimated leaf surface might be expected in comparing different species, but for any one species the root-leaf ratio appears to be a reliable quantity in comparing individuals.

Results are shown graphically in figure 1 where transpiration per unit of leaf area is plotted over root-leaf ratio. A comparison of root-leaf ratios (root dry weight divided by leaf area) with transpiration per unit of leaf area shows that an increase in root-leaf ratio is accompanied by an increased transpiration rate per unit of leaf area. Red pine, however, was a notable exception and showed a nearly similar transpiration rate with varying amounts of root weight and leaf area. This species was observed to have a relatively large, extensively branched root system and densely clustered needles. It seemed possible that with a small exposed

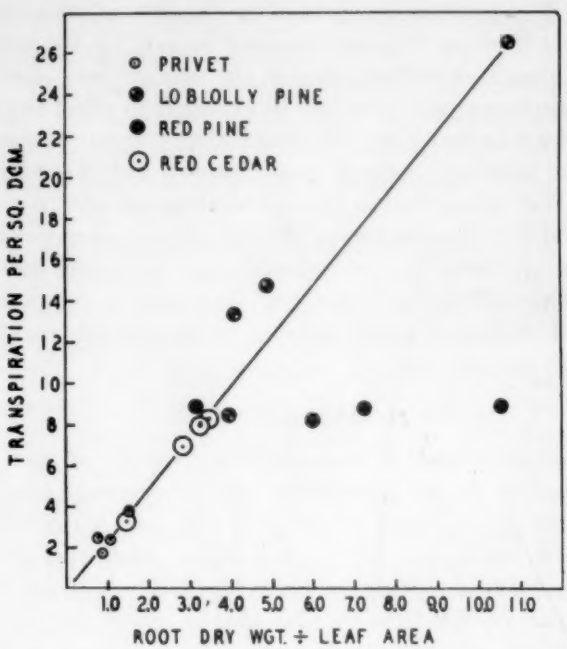

FIG. 1. Grams of water lost per square decimeter of leaf area per day is plotted over root dry weight in grams divided by leaf area in square decimeters. Leaf area was estimated as amount of leaf surface intercepting direct light.

leaf surface together with an extensive root system, transpiration of red pine was not limited by absorption in this experiment.

Assuming the relationship in figure 1 to have the form $Y = bx$, an analysis of variance for each of the species shows that this formula is not significant for red pine and privet but that it is significant for loblolly pine and red cedar at the 5 and 1 per cent. levels respectively.

In order to investigate further the effect of differences in the root-leaf ratio on the transpiration rate per unit leaf surface, one group of pine seedlings and one of red oak were pruned in such a way that there were 16 variations in root-leaf ratio for each species.

Sixteen northern red oak (*Quercus borealis*, var. maxima (Marshall) Ashe) seedlings with about seven leaves per plant were divided into four groups. The leaf surface of three groups was reduced to one, three, and five leaves respectively, and the fourth group left intact. The extent of the root system of three of the four plants in each group was then reduced by approximately 25, 50, and 75 per cent. and the root systems of the fourth group were left intact. Root pruning was accomplished by cutting off a section of the soil mass leaving the remaining roots undisturbed. This removed section was then replaced with soil of about the same texture in the process of transplanting to the containers. This gave 16 variations in root-leaf ratio with one plant entirely intact at

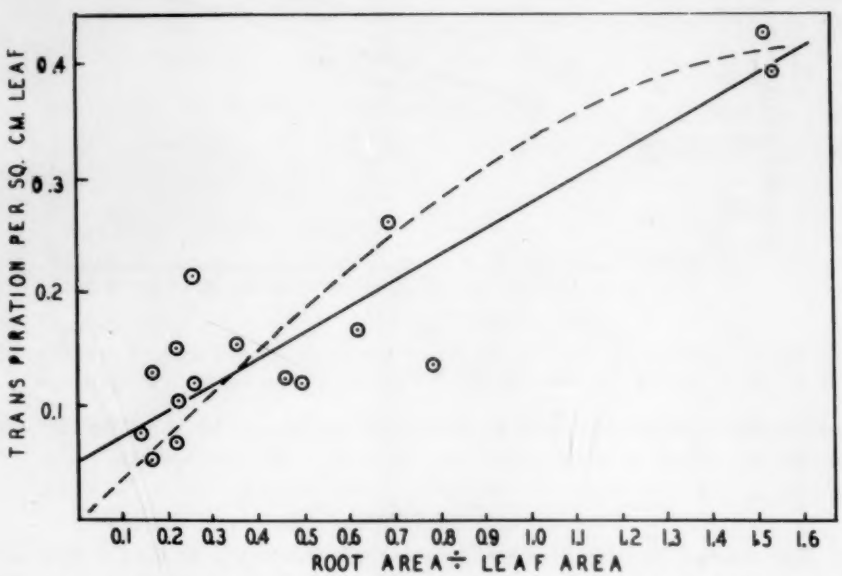

FIG. 2. Grams of water lost per square centimeter of leaf area of loblolly pine per day is plotted over root area divided by leaf area in square centimeters.

one extreme and one with leaf area and root systems severely reduced at the other. Transpiration rates were measured as before for one week and both leaf and root surfaces were estimated in the photoelectric apparatus. Crossing of small roots and leaves in the apparatus was inevitable but this method gave better results than those which were obtained when root dry weight and leaf volume were used for plotting curves similar to those in figures 2 and 3.

This experiment was then repeated with loblolly pine. An approximation of 25, 50, and 75 per cent. leaf area and root area reduction was made by ocular estimation. The leaf and root area remaining was measured in the photoelectric apparatus after completion of the experiment.

Results are shown graphically in figures 2 and 3 where increasing root-

leaf ratios are associated with increasing transpiration rate per unit leaf area. The solid lines show the relationship

$$\frac{T}{L} = a + b\frac{R}{L}$$

where T is transpiration, L leaf area, R root surface, and a and b are constants. For the two species this formula proved to be significant at the one per cent. level. It is more logical to suppose that the relationship has its origin at zero and tends to level off somewhat according to the dashed

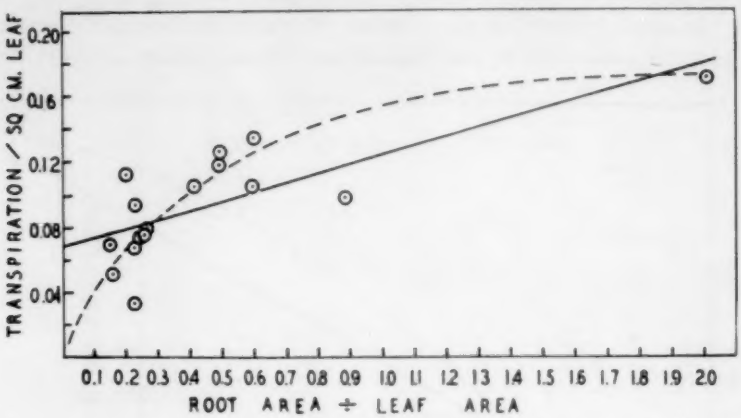

FIG. 3. Grams of water lost per square centimeter of leaf area of northern red oak per day is plotted over root area divided by leaf area in square centimeters.

lines shown in figures 2 and 3. However, the nature of the data did not justify the plotting of such a curve.

Discussion

The chief difficulty in experiments of this nature is to find a satisfactory basis for measuring the actual absorbing surface of the roots. In the first place, the entire root surface is not equally effective as a water absorbing surface. While most of the water intake probably occurs through the regions near the tips, some absorption apparently occurs through the older, suberized regions of woody roots (**4**). Since in dealing with entire root systems there is as yet no entirely satisfactory method for determining the relative importance of the regions of various ages in absorption, they were all combined. A second difficulty is the fact that root surface is not proportional to dry weight. Large roots possessing less surface area per unit of weight than small roots. In order to reduce the effect of this difficulty, root surface was measured in the same manner as the leaf surface in the second and third experiments. Because of these difficulties and also because of those caused by differences in leaf structure, it is unwise to make any strict comparisons between species in these experiments.

Conclusions

A study was made of the relationship between the rate of transpiration of a number of species of woody plants and the ratio of root to leaf surface.

Transpiration rate per unit of leaf area of loblolly pine and red cedar were found to increase as a function of the ratio of root surface to leaf surface. This relation was not proven to be significant in the case of privet and red pine, although privet conformed roughly to the line shown in figure 1.

When the ratio of root surface to leaf surface of loblolly pine and red oak seedlings was varied by removing various amounts of root and leaf surface, the water loss per unit of leaf surface was found to be significantly correlated to the ratio of root surface to leaf surface, as shown in figures 2 and 3.

The author would like to express his indebtedness to Professors Paul J. Kramer and Francis X. Schumacher for advice in connection with this study.

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BIOCHEMICAL CHANGES IN TERMINAL TUNG BUDS DURING THEIR EXPANSION PRIOR TO BLOSSOMING

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(WITH ONE FIGURE)

Received December 20, 1948

Introduction

For a number of years, the Bureau of Plant Industry, Soils, and Agricultural Engineering has conducted a broad program of research on the physiology of the tung tree, with the general objective of developing new tung varieties and cultural practices that will lead to increased production of tung oil. In the course of this research it was believed to be of value from the nutritional standpoint to determine the time at which biochemical changes associated with the breaking of dormancy in terminal tung buds are initiated and the kinds and amounts of organic and inorganic reserve materials accumulated by such buds from the time they begin to expand until the inflorescence is complete. An investigation was undertaken with these objectives in view, and the findings are reported in this paper.

The substances commonly found in plant tissues are carbohydrates, lipids, proteins, amino acids, organic acids and their salts, water, and inorganic compounds. In most biochemical studies of this nature the carbohydrate and nitrogen fractions have received the most attention, as illustrated by the work of HOWLETT (**4**) and MCDERMOTT (**8**), who reported marked changes in these fractions for apple and yellow poplar buds during their development. Although several investigators (**2, 5, 6, 7**) have noted that sterols in the unsaponifiable portion of the lipid fraction of seeds undergo significant changes during germination, changes in chemical composition of the lipid fraction of terminal buds during their expansion prior to blossoming appear to have received comparatively little study. Because of the high ether extract values in dormant tung buds, which material was found to constitute 34% of the dry weight of such buds in January, it was decided to determine whether any changes occurred in this fraction during the period of expansion of the terminal buds.

Experimental

For this study, four plots of tung trees in a 17-year-old orchard near Gainesville, Florida, were used. Each plot consisted of four trees growing under good cultural conditions. A composite sample of 100 terminal buds, 25 per tree, was taken from each plot on each of five dates: viz, January 15,

[1] Associate chemist (resigned) and junior plant physiologist (resigned), respectively, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture.

TABLE I

CHANGES** IN ORGANIC CONSTITUENTS OF TERMINAL TUNG BUDS DURING EXPANSION PRIOR TO BLOSSOMING

SAMPLING DATES	STAGE† OF DEVELOPMENT	CARBOHYDRATES					NITROGEN			LIPIDS		
		DRY MATTER	REDUCING SUGARS	NONREDUCING SUGARS	STARCH	POLYSACCHARIDES††	TOTAL NITROGEN	ALCOHOL SOLUBLE NITROGEN	ALCOHOL INSOLUBLE NITROGEN (BY DIFFERENCE)	CRUDE ETHER EXTRACT	UNSAPONIFIABLE FRACTION	FATTY ACIDS (BY DIFFERENCE)
		PERCENTAGE DRY WEIGHT										
Jan. 15	1	59.81	10.10	0.38	7.00	4.57	1.19	0.074	1.12	34.01	26.63*	7.38
Feb. 15	1	59.92	5.02	5.82	6.76	3.43	1.28	0.064	1.22	30.60	24.35*	6.25
Mar. 15	1	58.15	1.81	4.25	7.52	3.93	1.19	0.061	1.13	28.63	9.84	18.79
Mar. 25	2	61.75	0.68	0.53	4.29	2.62	2.06	0.118*	1.94	13.49	4.09	9.40
April 17	4–6	16.52*	4.62	0.25	11.12*	5.16	2.55	0.593*	1.96	10.54	2.49	8.05
LEAST DIFFERENCE SIGNIFICANT AT	.05	1.40	0.90	0.81	1.30	1.09	0.24	0.014	.....	3.29	2.91	.....
	.01	2.02	1.27	1.15	1.96	1.55	0.34	0.022	.....	4.61	4.83	.....
		AVERAGE MILLIGRAMS PER BUD										
Jan. 15	1	420.	42.2	1.6	29.4	19.3	5.0	0.3	4.7	143.	110.*	33.
Feb. 15	1	446.	22.3	25.7	30.2	15.8	5.7	0.3	5.4	137.	113.*	24.
Mar. 15	1	401.	7.3	17.0	32.3	15.5	4.7	0.2	4.5	115.	42.	73.
Mar. 25	2	667.	4.5	3.7	28.1	17.5	13.7	0.8	12.9	90.	27.	63.
April 17	4–6	1910.	87.8	4.7	212.0*	99.0*	48.7*	11.3*	37.4	201.	48.	153.
LEAST DIFFERENCE SIGNIFICANT AT	.05	47.	11.8	4.2	7.7	6.9	1.0	0.09	.....	24.	10.	.....
	.01	67.	16.6	5.9	11.7	10.2	1.4	0.14	.....	34.	17.	.....

* Not included in statistical analyses (see text).

** Each entry in the table is the mean of duplicate determination on four samples, one from each of four replicate plots.

† Stages as illustrated in figure 1.

†† Acid hydrolyzable residue from starch determinations.

February 15, March 15, March 25, and April 17 of 1942. All samples were taken by breaking or cutting the buds off just below the point of attachment of the bud scales.

Immediately after collection, the samples were taken to the laboratory and dried rapidly to constant weight over phosphorus pentoxide in a vacuum oven at 70° C and 4 mm. pressure. The samples were analyzed for alcohol-soluble nitrogen, reducing sugars, non-reducing sugars, starch, and polysaccharides[2] according to the methods outlined by SELL, JOHNSTON, and LAGASSE (**11**); and for total nitrogen, ether extract, and unsaponifiable matter by A.O.A.C. methods (**1**). The calcium, potassium, magnesium, and phosphorus were determined according to the procedure outlined by DROSDOFF and PAINTER (**3**). The results obtained are presented in tables I and II.

TABLE II

CHANGES** IN INORGANIC CONSTITUENTS OF TERMINAL TUNG BUDS DURING EXPANSION PRIOR TO BLOSSOMING

SAMPLING DATES	STAGE† OF DEVELOPMENT	ASH	CALCIUM AS Ca	MAGNESIUM AS Mg	PHOSPHORUS AS P	POTASSIUM AS K
		PERCENTAGE DRY WEIGHT				
Jan. 15	1	4.19	1.02	0.28	0.017	0.46
Feb. 15	1	4.08	0.96	0.28	0.018	0.48
Mar. 15	1	4.24	0.96	0.27	0.019	0.51
Mar. 25	2	2.95	0.49	0.17	0.022	0.58
April 17	4–6	6.94	0.64	0.27	0.041*	1.46*
LEAST DIFFERENCE SIGNIFICANT AT	.05	0.42	0.16	0.03	0.001	0.80
	.01	0.60	0.22	0.04	0.002	1.14
		AVERAGE MILLIGRAMS PER BUD				
Jan. 15	1	17.6	4.3	1.2	0.07	1.9
Feb. 15	1	18.2	4.2	1.2	0.08	2.1
Mar. 15	1	16.9	3.6	1.1	0.08	2.0
Mar. 25	2	19.7	3.2	1.1	0.15	3.9
April 17	4–6	132.8*	12.2*	5.2*	0.78*	27.9*
LEAST DIFFERENCE SIGNIFICANT AT	.05	1.8	0.8	0.1	0.01	0.5
	.01	2.6	1.2	0.2	0.02	0.8

* Not included in statistical analyses (see text).

** Each entry in the table is the mean of duplicate determination on four samples, one from each of four replicate plots.

† Stages as illustrated in figure 1.

Presentation and discussion of results

In mature tung trees lateral buds ordinarily remain dormant, and all growth is made from the terminal buds which, with rare exceptions, are mixed buds containing both embryonic flowers and vegetative growing points. The growth that took place during the sampling period was arbitrarily divided into the six stages that are illustrated in figure 1. On the

[2] Acid hydrolyzable residue from starch determinations.

first three sampling dates, the buds were all in stage 1, on March 25 they were mostly in stage 2, and on April 17 they were in stages 4, 5, and 6, with stage 5 predominating. Rapid changes in the morphology of the tissues took place during the transition from stage 1 to stage 6. In January the buds consisted largely of stem tissues including undifferentiated meristem and differentiated pith, xylem, cortex, and phloem, with rudimentary flowers and leaves enclosed in bud scales. During the dormant season the embryonic flower buds of tung were embedded in a sticky wax which disappeared as the buds attained stages 5 and 6. As growth began a short axis developed and served as a base for the inflorescence. This axis or flower base consisted largely of pith tissue within a thin cylinder of woody tissue

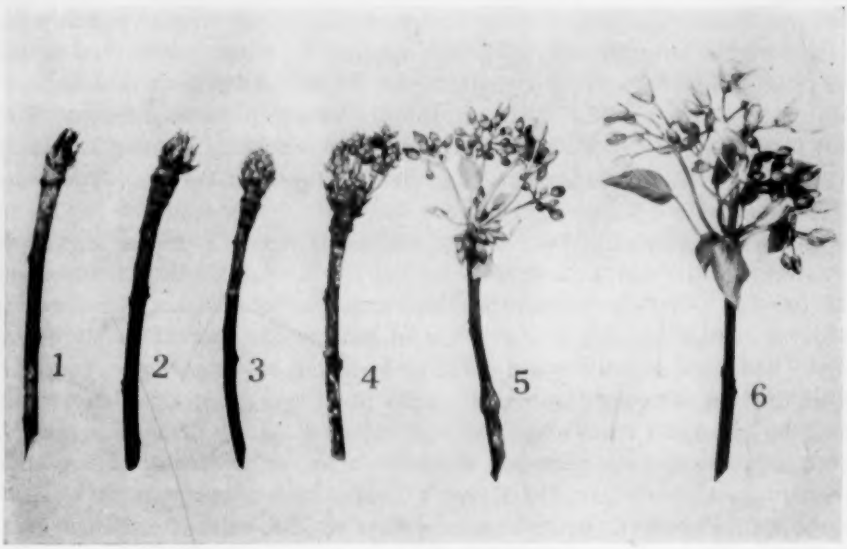

FIG. 1. Terminal buds from shoots of the tung tree, illustrating 6 stages of expansion.

and immature bark. The flower buds present at the close of the dormant period developed into the inflorescences consisting of peduncles, pedicels, and blossom buds, all of which were composed of very succulent tissues. Leaves were few in number and remained very small until stage 5. Thus, in the early portion of the sampling period the tissues of the bud were largely mature and woody; at the later sampling they were largely immature and succulent.

Inspection of the data in table I shows that values for most of the constituents differed widely between samples collected on certain dates. For example, between January 15 and March 25 the percentage of dry matter ranged from 58.15 to 61.75, but on April 17 a marked decrease to 16.52 was observed. No statistical analysis is necessary to show that a very significant change took place between March 25 and April 17. Furthermore, if statistical methods are used to determine the significance of the variations in per-

centage of dry matter during the first four sampling dates, it is evident that the values for the four replicate samples collected on April 17 differ so widely from those collected earlier that they should not be included in the same analysis of variance. Accordingly, in the several statistical analyses reported, only the data for those samples in which the significance of the trends was not self-evident have been used.

Between January 15 and February 15 biochemical changes occurred, although there was no visible expansion of the buds. There was no significant change in the dry weight of the buds during the first three sampling periods, but after March 15 and especially after March 25 the weight increased rapidly due to the greatly accelerated growth of buds into the more advanced stages of development, 4, 5, and 6, as illustrated in figure 1. Small but statistically significant changes in percentage of dry matter, possibly due to weather, occurred during the first four sampling dates. A marked change in relationship between dry matter and water content occurred between March 25 and April 17. Although the dry weight per bud increased from 667 to 1910 mg., the percentage of dry matter decreased from 61.75 to 16.52. This change was due to the normal growth processes of the buds from stage 2 to the advanced stages 4, 5, and 6.

As the buds developed into stage 2, the milligrams of reducing sugars per bud decreased from 42.2 on January 15 to 4.5 on March 25. Between January 15 and February 15 there was a gain of 24.1 mg. of non-reducing sugars per bud, and a loss of 19.9 mg. of reducing sugars. Possibly hexose sugar was converted into a non-reducing form, but reducing sugars may have been utilized in respiration and the gain in sucrose effected through translocation of sugars from other parts of the tree. A significant decrease in both reducing and non-reducing sugars occurred before visible expansion of the buds took place, that is by March 15, which corresponds with the observations of McDermott (**8**) on buds of yellow poplar. Between February 15 and March 25, the non-reducing sugars of the tung bud decreased on the average from 25.7 to 3.7 mg. while the percentage decreased from 5.82 to 0.53. During the same period there were no significant changes in starch and polysaccharides on a weight per bud basis, but a decrease in percentage of these constituents occurred as the buds passed from stage 1 into stage 2 (March 15 to March 25). On a weight per bud basis, reducing sugars, starch, and polysaccharides increased rapidly from March 25 to April 17, as the buds approached the blossom stage (from stage 2 into stages 4, 5, and 6). An increase in the reducing sugars, total sugar, and polysaccharides of apple buds during a similar period has been observed by Howlett (**4**). Only a small part of the large increase of hexose sugar can be accounted for by photosynthesis since only a few leaves had formed by April 17 (stages 5 and 6). Sell, Best, and Lagasse (**10**) have shown that starch and sucrose accumulate in the roots and the twigs of the tung tree during dormancy. When the tree resumes growth in spring, these carbohydrates could be in part converted into sugars and translocated to growing points. The re-

ducing sugars and starch accumulated in the flower-bearing axis must have come from some such source. Although, as was previously pointed out, the expansion of the inflorescence involves rapid growth and a high rate of metabolism, a still higher rate of growth and metabolism must take place immediately following the expansion of the inflorescence when leaves, shoots, and fruit develop. Some of the sugar and starch accumulated in the inflorescence at blossom time may later be utilized in the production of lipids, protein, and other constituents of the new protoplasm and structural tissues.

Between January 15 and March 15 there were but slight fluctuations in the total and the alcohol-insoluble nitrogen fractions (table I) either on a percentage or on a milligram per bud basis, while in the alcohol-soluble fraction the data suggest a downward trend. McDERMOTT (**8**) reported a decrease in the percentage of total and insoluble nitrogen for a similar period in yellow poplar buds. The data indicate a marked increase in protein nitrogen, determined as insoluble nitrogen, both on a weight and on a percentage basis between March 15 and 25 (stages 1 and 2) when a corresponding loss in carbohydrates and lipids occurs. McDERMOTT (**8**) observed the same trends in percentage of insoluble nitrogen of poplar buds. In general the total nitrogen in the tung bud increased gradually as the unexpanded buds developed to near full bloom (stages 4, 5, and 6) and these data are in agreement with the work of HOWLETT (**4**) on apple buds. It is evident also that the weight of insoluble and soluble nitrogen fractions increased from 12.9 to 37.4 mg. and from 0.8 to 11.3 mg., respectively, as the buds developed from stage 2 into stages 4, 5, and 6. The increase in the insoluble fraction is probably due to the synthesis of protein nitrogen from soluble nitrogenous compounds translocated into the buds (**9**) from older twigs and branches. Protein nitrogen stored in the bud in this stage could serve probably as a source of reserve nitrogen for protoplasm of new growth.

The lipid fraction (ether extract) and unsaponifiable matter on a milligram per bud and a percentage basis followed approximately the same trend during expansion of the bud as did most of the carbohydrates (table I). The greatest changes in the lipids were found to occur in the unsaponifiable fraction. Between February 15 and March 15, before any appreciable expansion of the buds took place, the unsaponifiable matter decreased sharply on both weight per bud and percentage basis and this trend continued as the buds passed from stage 1 into stage 2. No loss of waxy scales was observed when the buds expanded during these stages. The fact that this fraction decreased in weight so markedly during growth indicates that it may have a metabolic role. An increase in the weight of unsaponifiable matter occurred as the buds in stage 2 developed into stages 4, 5, and 6. The increase may consist of lipids occurring as constituents of the protoplasm formed in the new growth. From these data it is apparent that the changes in the unsaponifiable matter may be closely associated with physiological activity. In the soybean, a similar relationship between the metabolism of sterols in the unsaponifiable fraction and germination was observed by MACLACHLAN

(**6, 7**). In the early stages of bud expansion, the fatty acids from ether extract did not show the same trend as the unsaponifiable matter. The first significant change of this fatty acid constituent was a 201 per cent. increase on a percentage basis and a 204 per cent. increase on a milligram per bud basis, occurring between February 15 and March 15. An increase of 90 mg. per bud occurred when the buds passed from stage 2 (March 25) into stages 4, 5, and 6 (April 17). At this stage the fatty acids accumulated to a greater extent than did the unsaponifiable matter.

The ash content of the buds on a per bud and on a percentage basis (table II) remained relatively constant during the first three sampling periods (stage 1). The same was true of calcium, magnesium, phosphorus, and potassium. Between March 15 and April 17, as the buds passed from stage 1 through stage 2 into stages 4, 5, and 6, the dry weight per bud increased 376 per cent., while the quantity of ash per bud increased 686 per cent. This shows that there is a movement of these inorganic materials from other parts of the tree to the buds. The data in table II show that during the development of the buds from March 25 to April 17, the weight per bud of calcium increased 281 per cent., that of magnesium 373 per cent., of phosphorus 420 per cent., and potassium 615 per cent. Meristematic tissues are characteristically rich in potassium. The data show that substantial amounts of inorganic nutrients are required during the period of expansion of the tung buds.

Summary

1. The biochemical changes occurring in the tung bud late in the dormant period and during the period of rapid expansion of the inflorescence were studied.

2. The reducing sugars decreased both in milligrams per bud and in percentage from late winter until just prior to the expansion of the blossom cluster; non-reducing sugars increased at first, then followed the same general trend as reducing sugars. Starch and polysaccharides remained relatively constant during the early stages. During the period of rapid bud expansion all carbohydrates increased markedly, except non-reducing sugars which increased only slightly in milligrams per bud and decreased on a percentage basis. Since at the end of the study leaves were just beginning to develop, practically all the carbohydrates that accumulated in the new growth must have been translocated from storage tissues in the tree.

3. The nitrogen fractions fluctuated slightly on a milligram per bud and a percentage basis early in the period of sampling. The alcohol-soluble fraction, representing the soluble nitrogenous products, increased as the buds approached the blossom stage. A similar trend was noted in the alcohol-insoluble fraction on a weight per bud basis. The increase of the latter fraction was probably due to the synthesis of protein nitrogen from the soluble nitrogenous compounds translocated from storage tissues of the tree for the production of the new growth.

4. The lipid fraction (ether extract) expressed either on a milligram per bud or on a percentage basis decreased gradually until just before the period of rapid expansion of the inflorescence. The important changes in amount of ether extract occurred mostly in the unsaponifiable fraction, which may be physiologically active. In the later stages, just before flowering, the increase in the weight of fatty acids per bud was much more pronounced than the increase in the unsaponifiable matter.

5. The ash content expressed either on a per bud or on a percentage basis remained almost constant during the early periods of sampling, but increased rapidly just prior to flowering. The same trend was observed for the calcium, magnesium, phosphorus, and potassium. Without a doubt a large proportion of the inorganic nutrients, such as calcium, magnesium, and phosphorus, was assimilated as constituents of the protoplasm and the cell walls. The data show that the requirements for these inorganic nutrients during expansion of tung buds were substantial.

The authors are indebted to Dr. Matthew Drosdoff for making the mineral determinations and to Dr. George F. Potter for assistance with the statistical analyses and in the preparation of this manuscript.

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EFFECTS OF PHOTOPERIOD AND NITROGEN SUPPLY ON GROWTH AND REPRODUCTION IN THE GHERKIN

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(WITH SIX FIGURES)

Received June 1, 1948

In view of the fragmentary knowledge of and growing interest in the ontogeny of cucurbits, an experiment was undertaken to trace certain metabolic and formative processes during the life cycle of a representative species of this family. Though many species of cucurbits are known to be day-neutral in respect to flowering, their growth and certain aspects of reproduction are, nevertheless, known to be highly responsive to day length and nitrogen supply (**10, 11, 12, 38**). Hence, contrasted conditions of day length and nitrogen nutrition were employed in the experiment in order to identify as far as possible plant responses associated with each of these variables. The common gherkin, *Cucumis anguria* (**2**), was selected for the experiment on the basis of the essentially day-neutral character of its flowering response, convenience of plant size and brevity of its life cycle. The development and growth of vegetative and reproductive organs, as well as the composition of plants and substrate, was observed from the early vegetative phase to maturity.

Experimental Methods and Procedure

Seedlings of the commercial strain of the small gherkin, *Cucumis anguria*, were transplanted into one-gallon glazed jars containing a uniform weight of quartz gravel prior to the emergence of the first true leaf.

The plants were then divided into two experimental groups, each consisting of 40 plants, grown in diurnal photoperiods of eight and 16 hours. The short-day plants received normal daylight from 8 A.M. until 4 P.M. The long-day plants were exposed to normal daylight plus artificial light as necessary from a battery of 40-watt fluorescent "day-light" lamps with an intensity of approximately 200 foot candles at the bench level.

The plants were cultured on a nutrient solution containing the salts indicated (table I) plus traces of iron, boron, manganese, and copper. All plants were started on an initial control solution of medium nitrogen content. Upon initiation of growth following transplanting, the nitrogen supply of both long- and short-day plants was altered by withdrawal of nitrogen from one half the plants in each series and increasing available nitrogen in the other half as indicated (table I).

Nutrient solutions were replenished weekly and solution residues were preserved for chemical analysis. The pH of the original nutrient solution and residues was determined electrometrically. Diurnal and nocturnal

temperatures of 80° and 70° F were maintained thermostatically. Special precautions were taken to prevent nocturnal temperatures below 70° as SCHROEDER (**33**) has shown detrimental effects of low soil temperatures in the water relations of the cucumber.

Weekly measurements of stem elongation, number of nodes and length of internodes, number of leaves and areas, and number of flowers were recorded. Tissues were dissected to observe microscopically the time of initiation of floral primordia, synapsis, sporogenesis, gametogenesis, and syngamy. One half of the plants of all series were sampled for chemical analysis at full anthesis (approximately 65 days of age) and the remainder at the mature fruit stage (age 108 days), samples being collected at the end of the daily dark period. Fresh weight of roots and shoots was determined and the parts then comminuted for analysis. Fifty gram samples were preserved in boiling 80 per cent. alcohol for carbohydrate determinations according to official methods (**1**). The material preserved in alcohol was extracted several times in 80 per cent. alcohol, allowed to dry, finely ground, and the extraction completed by refluxing in a Soxhlet apparatus (**24**). Aliquots of the clarified extract were hydrolyzed with concentrated hydrochloric acid for the determination of soluble sugars by a modified micro-method of FOLIN and MALMROS (**4**) and the results expressed as dextrose. The residue from the alcoholic extraction was hydrolyzed for three hours with dilute hydrochloric acid. Triplicate samples were weighed for dry weight in a Brabender moisture tester. Material for nitrogen and ash determinations was rapidly dried in a forced draft oven according to the method of LOOMIS and SHULL (**24**). Dried tissue ground to 60 mesh was used for determination of total nitrogen including nitrates by the micro-Kjeldahl method of KOCH and MCMEEKIN (**18**) which has been found appropriate for plant tissue (**8, 20, 42**). Soluble nitrogen was extracted from finely ground dried material and the total soluble nitrogen in the extract determined as for total nitrogen. Insoluble nitrogen was recorded by difference. Ash was determined by slow ignition of dried tissue at red heat over a Bunsen flame.

TABLE I

CHEMICAL COMPOSITION OF NUTRIENT SOLUTIONS EMPLOYED
COMPONENTS GIVEN IN PARTS PER MILLION IN DISTILLED WATER

C. P. SALT	CONTROL SOLUTION	LOW N SOLUTION	HIGH N SOLUTION
KNO_3	142	.....	142
KH_2PO_4	142	142	142
$MgSO_4 \cdot 7H_2O$	142	142	284
$Ca(NO_3)_2 \cdot 4H_2O$	568	.....	568
KCl	.....	105	.....
$CaCl_2$	.....	390	.....
NH_4NO_3	.....	.....	1165

Results

Gross Morphology

Habit.—For a period of about 20 days after transplanting, rates of growth in all series were fairly uniform. The plants were healthy, dark green in color except in the low nitrogen plants which began to manifest typical nitrogen deficiency of the leaves. Root growth was uniform and appeared normal throughout. Stem growth followed a fairly uniform pattern of elongation in all variables until the inception of flower primordia.

Stems.—Upon initiation of flower buds, distinct differences in rate of elongation and in total stem height became apparent (table II, figs. 1 and

TABLE II

Average number of nodes per plant and average internodal length

Age in days	Growth phase	Short day				Long day			
		High N		Low N		High N		Low N	
		Nodes no.	Length cms.	Nodes no.	Length cms.	Nodes no.	Length cms.	Nodes no.	Length cms.
10	Veg.	1	2.00	1	2.00	1	2.00	1	2.00
17	Veg.	2	2.00	2	2.00	2	2.00	2	2.00
24	Veg.	3	2.26	3	2.00	3	2.00	2	2.00
31	Bud.	5	2.50	7	1.15	4	2.00	4	1.50
38	Bud.	8	3.15	8	1.80	7	1.25	6	1.50
45	Flow.	13	2.90	10	2.64	9	1.44	7	1.43
52	Flow.	18	2.88	15	2.73	12	1.54	8	1.62
59	Flow.	23	3.00	20	2.40	15	1.81	9	1.72
66	Flow.	24	2.92	21	2.43	16	2.81	11	2.27
73	Flow.	28	3.14	22	2.63	17	3.94	11	2.94
80	Flow.	31	3.32	23	2.91	20	4.25	13	2.80
87	Fruit.	36	3.05	23	2.95	22	5.45	15	2.67
94	Fruit.	41	3.12	24	2.87	26	6.19	15	2.93
101	Fruit.	43	3.02	25	2.80	31	5.29	16	2.93
108	Fruit.	43	3.04	25	2.80	32	5.18	16	3.00
108	Total Stem Length		131.00		70.00		166.00		48.00

3). Long-day, high-nitrogen (LD-HN) plants eventually exceeded those in short day, high nitrogen (SD-HN) in stem length chiefly by rapid elongation of internodes, although rapid extension of the main axis under long-day conditions did not begin until late in anthesis (fig. 1). On the other hand, stem length of short-day and low-nitrogen (SD-LN) plants was consistently greater than in long-day, low-nitrogen (LD-LN) plants (fig. 1). Low nitrogen interfered with stem elongation by reduction in number of nodes and length of internodes (9) (table II). Stems of short-day plants produced more nodes than those in long day.

Leaves.—At 30 days of age no appreciable differences in the external structure of the leaves of the various series were noted except that the foliage of the low nitrogen plants was light green in color (fig. 3). There-

after, however, distinctive contrasts in foliar morphology became evident. Leaves of the LD-HN plants were markedly pubescent and the shield-shaped laminae tended to bend downward from the petiole (fig. 3). Foli-

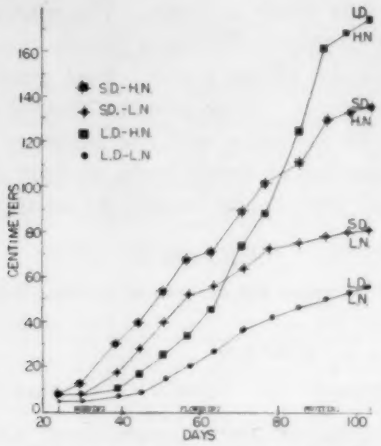

FIG. 1. Average stem length per plant during growth.

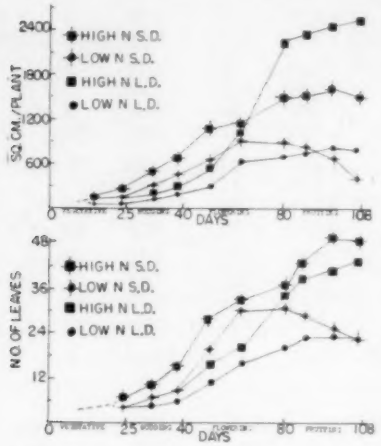

FIG. 2. Average number of leaves (below) and average leaf areas in square centimeters per plant (above).

age of this group also exhibited a glossy, dark green color while those under comparable short-day (SD-HN) conditions had a light green hue, were thinner and less pubescent. The basal leaves of the low-nitrogen plants in both day lengths were chlorotic, white to lemon colored, thin,

FIG. 3. Representative plants photographed at various growth stages. A: 40 days of age, late budding stage. B: 60 days of age, anthesis. C: 80 days of age, late flowering, early fruiting stage. D: 95 days of age, late fruiting stage. Left to right: 1. short day, high nitrogen, 2. short day, low nitrogen, 3. long day, high nitrogen, 4. long day, low nitrogen.

D
C
B
A
1
2
3
4

and relatively stiff. The acute angle of insertion of the petiole with the stem and the upward bending of the lamina to form a V-shaped position with the midrid were very characteristic (fig. 3). Leaves of the short-day plants were smaller but more numerous than those of the long-day plants. Total leaf area per plant was much greater in the long day (fig. 2). Considerable foliar abscission occurred in plants exposed to the short photoperiod. High-nitrogen plants produced more leaves and a considerably greater foliar surface (fig. 2) than the low-nitrogen plants (**40**). Rapid increase in leaf area was correlated with relatively low formation of new leaves in all series during the blossoming period (fig. 2).

Roots.—Roots of long-day plants observed at the two sampling dates were consistently larger than those of short-day plants and were profusely branched (**11**).

Flowers.—Although flower bud formation was initiated simultaneously in plants of both long- and short-day series, plants varied appreciably in flower number as well as in date and duration of the period of maximal bloom (table III). The peak of flower production occurred 15 days earlier

TABLE III

Average total weekly number of staminate and pistillate flowers per plant (Each week's count represents all intact flowers both old and newly formed.)

Age in days	Short day				Long day			
	High N		Low N		High N		Low N	
	Male	Female	Male	Female	Male	Female	Male	Female
43	5	0	2	0	3	0	1	0
50	11	2	5	0	3	0	1	0
57	17	3	9	1	7	1	3	0
64	34	5	16	2	12	3	8	1
71	39	6	23	3	16	6	9	1
78	46	7	26	3	31	7	12	2
85	50	8	28	4	33	7	13	2
92	62	10	31	4	35	8	15	3
99	58	12	30	5	37	8	18	3
106	55	12	30	5	39	8	18	3
Total per Plant	67.0		35.0		47.0		21.0	
Ratio Male/Female	4.58		6.0		4.87		6.0	

in the eight-hour day length and approximately 50 per cent. more flowers were formed than in the long-day plants. In agreement with data of other workers (**12, 23, 37, 38**), more pistillate flowers were produced by the high-nitrogen plants in both photoperiods (table III).

Nutrient Solution Data

Water Absorption.—Though high-nitrogen plants consumed a greater total amount of water than the low-nitrogen groups and long-day plants

more than plants in short day, these absorption curves were essentially parallel during the growth cycle (fig. 4). Water absorption was essentially comparable among the experimental groups until the time of floral inception. After flowering, however, water absorption in the long-day plants gradually surpassed that of short-day groups. As observed by DENNISON (**13**) working with tobacco, the gherkin also shows a sharp reduction in water intake just prior to or coincident with both synapsis and syngamy. The sharp decline in water absorption associated with the appearance of flower primordia in most cases reached a steady but low rate

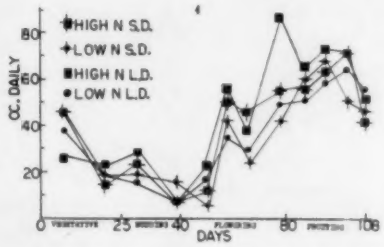

FIG. 4. Average daily water absorption in cubic centimeters per plant.

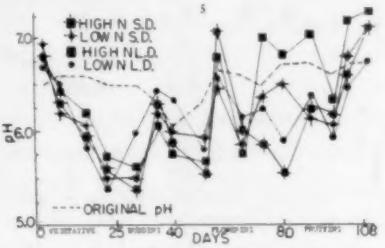

FIG. 5. pH changes of nutrient solution residues compared to pH of original nutrient solution.

thereafter until the onset of sporogenesis. A temporary acceleration in the rate of water absorption occurred at the inception of microsporogenesis (approximately 29 days of age) and megasporogenesis (approximately 39 days of age), which though moderate in amount, seemed characteristic and probably significant to judge from its consistency in all plants of a given series. A second reduction in the rate of water absorption was noted prior to and in association with syngamy (aproximately 65 days of age), followed in turn by a marked secondary increase during early stages of fruit enlargement. Similar minima and maxima of water consumption have been previously observed by the author in buckwheat (**17**) and by various workers using other species (**3, 21, 27, 30, 32, 35, 36, 41**).

NUTRIENT SOLUTION PH.—Alterations in pH of the nutrient solutions employed seemed to be correlated with fluctuations in the rate of water and salt absorption and with certain stages of the life cycle (fig. 5). There

was a fairly uniform decrease in the pH of the nutrient solution during the early vegetative development of all plants. Until the time of inception and differentiation of floral buds, pH values of the nutrient residues either remained unchanged or increased relatively slowly. Following flower emergence, however, hydrion concentration of nutrient residues increased appreciably. With the onset of maturity and senescence, pH values rose abruptly in all series (fig. 5). These minima and maxima of pH appear to be definitely associated with phases of ontogeny characterized respectively by low and high anabolic activity.

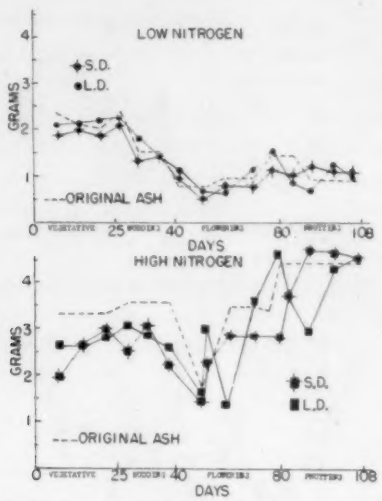

FIG. 6. Ash analysis of nutrient solution residues in grams per culture compared to original ash. Above: average of low nitrogen plants. Below: average of high nitrogen plants. Curves above the original ash line indicated higher ash content in solution residues than in original nutrient solution.

SALTS.—Mineral absorption was low or in some cases even negative during periods of minimal pH change in the nutrient solution residues. Periods of rapid growth and nutrient absorption were consistently correlated with a reduction in acidity of the substrate. Comparable trends in hydrion values of the media during ontogeny have also been noted for buckwheat (**17**) and hemp (**31**) maintained at uniform levels of nutrient supply.

On the basis of analyses of ash in solution residues, the minimal and maximal rates of salt absorption by the various plant groups show a correlation with: (a) minima and maxima of water intake, (b) pH fluctuations of nutrient residues, (c) the general growth rate, and (d) the developmental stage (figs. 4, 5, 6). Salt intake was rapid during active vegetative growth in both high- and low-nitrogen plants though actual values were less in plants of the low nitrogen series. The differentiation of floral structures apparently induced systemic changes in the plant to judge

from reduced rates of mineral intake. In some cases, the plants were actually losing salts to the substrate (fig. 6) as evidenced by a greater concentration of ash in the residues than present in the original nutrient solution (**7, 21**). In short day, salt absorption was greater in the early ontogenetic stages, but the converse relationship prevailed after flowers had emerged. Diminished intake or actual salt excretion just preceded or accompanied syngamy in all groups even though these phenomena occurred at different dates in the long- and short-day series (fig. 6). All plants showed increased rates of nutrient uptake and temporary restoration of nutrient balance following syngamy. Diminished absorption again occurred during the late stages of fruiting and leaching of salts from the roots marked the final phases of ontogeny, shown by larger amounts of solutes and ash in solution residues as compared with the initial salt content of the nutrient solution.

Data on Plant Composition

The long-day plants had produced greater fresh and dry weights than short-day plants at both sampling dates (table IV). Top-root ratios were smaller in long than in short day. Comparatively low top-root ratios in the low-nitrogen plants indicate that shoot growth was more adversely affected than root growth when the nitrogen supply was low. Data for total ash presents a somewhat complex picture in terms of amounts and distribution between root and shoot. Most of the ash was found in the shoots of plants in both photoperiods. In low-nitrogen plants, a larger fraction of the total ash content occurred in the roots (table IV). In the 43-day interval between analyses, short-day plants showed a decrease in actual ash content whereas total ash increased in long-day plants, achieving a fourfold gain in the LD-HN plants.

Determination of tissue moisture revealed that in all cases the roots gained in moisture content from anthesis to maturity whereas the shoots exhibited a reduction in moisture content (table IV). LD-HN plants elaborated greater actual amounts of soluble sugars and hydrolyzable polysaccharides than SD-HN plants at both sampling dates (table V). In terms of percentage of total dry weight, however, the SD-HN plants showed a higher relative fraction of total carbohydrates at maturity. At anthesis in the low-nitrogen groups, long-day plants contained more carbohydrates than the short-day plants but a converse relationship existed at maturity. Between the 65- and 108-day samplings, the SD-LN plants gained in percentage of hydrolyzable polysaccharides, an observation in agreement with other investigators (**6, 12, 19, 26**) who conclude that low nitrogen in general favors the accumulation of polysaccharides. In long day, however, the data on polysaccharide percentage are in accord with the work of Culpepper (**10**) with summer squash who found that hydrolyzable polysaccharides decreased with age in all parts of the plant except the fruit. In

TABLE IV

AVERAGE FRESH, DRY, AND ASH WEIGHT IN GRAMS PER PLANT; PERCENTAGE DRY WEIGHT AT THE TWO DATES OF SAMPLING

FRACTION	SHORT DAY						LONG DAY					
	HIGH N			LOW N			HIGH N			LOW N		
	ROOT	SHOOT	T/R	ROOT	SHOOT	T/R	ROOT	SHOOT	T/R	ROOT	SHOOT	T/R
	AT 65 DAYS OF AGE											
Fresh Weight, gms.	2.30	20.70	9.00	1.53	7.98	5.21	4.80	22.75	4.74	2.60	12.10	4.65
Dry Weight, gms.	0.35	2.68	7.68	0.40	1.54	3.85	0.60	3.01	5.01	0.50	1.77	3.54
Ash Weight, gms.	0.08	0.58	.....	0.15	0.46	.....	0.19	0.50	.....	0.12	0.29	.....
Dry Weight, %	15.00	12.94	.....	26.10	19.30	.....	12.40	13.20	.....	19.23	14.60	.....
	AT 108 DAYS OF AGE											
Fresh Weight, gms.	3.40	29.80	8.76	1.68	11.56	6.88	10.00	60.10	6.01	2.90	19.18	6.61
Dry Weight, gms.	0.37	5.30	14.30	0.41	2.55	6.22	1.10	9.08	8.18	0.52	3.91	7.51
Ash Weight, gms.	0.04	0.51	.....	0.13	0.38	.....	0.20	2.32	.....	0.16	0.42	.....
Dry Weight, %	10.88	17.78	.....	24.40	22.10	.....	11.10	15.10	.....	17.90	20.40	.....

cucurbits it thus seems that the effect of low nitrogen upon polysaccharide accumulation is largely conditioned by length of day (table V).

SD-HN plants attained a higher percentage of total nitrogen than LD-HN plants at both sampling dates (table V). At anthesis of high-nitrogen plants in long and short day, the percentage of total nitrogen was comparable but had decreased markedly at maturity of long-day plants. Total nitrogen increased progressively with age in all groups except the LD-LN plants (**12**). Most of the total nitrogen was in the soluble form in all

TABLE V

CARBOHYDRATES AND NITROGEN AS MILLIGRAMS PER PLANT AND PER CENT. DRY WEIGHT AT TWO DATES OF SAMPLING

FRACTION	SHORT DAY				LONG DAY			
	HIGH N		LOW N		HIGH N		LOW N	
	MGS.	%	MGS.	%	MGS.	%	MGS.	%
	AT 65 DAYS OF AGE							
Soluble Sugars	52.90	1.75	32.80	1.69	99.59	2.75	45.93	2.02
Polysaccharides	564.38	18.60	230.60	11.88	662.00	18.30	351.85	15.50
Total Carbohydrates	617.28	20.35	263.40	13.57	761.59	21.05	397.78	17.52
Soluble Nitrogen	26.40	0.87	7.76	0.40	21.30	0.59	10.67	0.47
Insoluble Nitrogen	20.70	0.68	2.13	0.11	13.72	0.38	2.50	0.11
Total Nitrogen	47.10	1.55	9.89	0.51	35.02	0.97	13.17	0.53
	AT 108 DAYS OF AGE							
Soluble Sugars	97.96	1.72	51.00	1.72	249.56	2.45	59.07	1.33
Polysaccharides	703.08	12.40	489.90	16.55	1002.66	9.85	408.18	9.22
Total Carbohydrates	801.04	14.12	540.90	18.27	1252.22	12.30	467.25	10.55
Soluble Nitrogen	64.07	1.13	12.07	0.40	72.28	0.71	11.32	0.26
Insoluble Nitrogen	52.16	0.92	8.52	0.29	115.03	1.13	2.77	0.06
Total Nitrogen	116.23	2.05	20.59	0.69	187.31	1.84	14.09	0.32

groups at 65 and 108 days except that insoluble nitrogen predominated in mature LD-HN plants (table V).

Discussion

If the foregoing data are viewed as a whole, the temporary but marked transition in type and amount of vegetative activity associated with flowering appears consistent and significant. Despite differences in degree and duration of response due to contrasts in nitrogen supply and daylength, the flowering phase in all series was characterized by a subsidence in the rate of differentiation of new leaves and nodes and by acceleration in the rate of foliar expansion and internodal elongation (figs. 1, 2). Long-day plants exhibited increase in foliar area rather than in leaf number whereas the converse seemed true in short day especially at high-nitrogen levels.

On the whole, flowering was also a period of low water and salt intake and of fairly stable but relatively low pH of the nutrient substrates. The

post-syngamic resurgence in production of new nodes and leaves was quite pronounced in the early stages of fruit formation. Enlargement and differentiation of new structures gradually subsided, however, as fruit enlargement accelerated.

Stem length was greater at maturity under long-day than under short-day conditions, a response also noted by MURNEEK (**23, 29**) in soybean and rudbeckia and by WITHROW (**40**) in a number of other species. Rapid extension of the main axis of the 16-hour plants occurred relatively late in ontogeny as also observed by DANIELSON (**11**). DEARBORN (**12**) and WITTWER (**41**) also noted rapid increment in the rate of stem elongation during inception of floral primordia and syngamy in the cucumber. The fact that alterations in growth rate were apparent in both photoperiods strongly suggests the influence of autogenous regulatory factors which are for certain periods and to a considerable degree largely independent of the edaphic and climatic variables. Some support for this conclusion may be derived from the marked variations in salt and water absorption which occurred during growth despite the fact that plants in each series were maintained at an essentially constant rate of nutrient supply by weekly renewal of the nutrient solutions.

In the light of the work of LOOMIS and FRANCO (**25**) indicating the ability of moderate amounts of KH_2PO_4 to cause iron chlorosis, it might appear that the foliar chlorosis of the low-nitrogen plants of the present study was due to similar conditions. Although this factor may have accentuated chlorosis in the low-nitrogen plants, it is not believed to be the major cause because chlorosis first occurred in the basal leaves and gradually progressed to higher leaves with accentuation of nitrogen deficiency, indicating that increasing severity of nitrogen deficiency rather than lack of iron in the plant was responsible for chlorosis in the gherkin. The retarding effect of short days upon root growth of several different genera noted by DANIELSON (**11**), DENNISON (**13**) and WEAVER and HIMMEL (**39**) was also observed in this investigation.

The fluctuations in water absorption, pH, and salt content of culture residues noted might at first appear to be fortuitous and perhaps ascribable to variations in temperature, light and nutrient solution composition. This conclusion seems unwarranted in view of the fact that temperature and the composition and intensity of light were uniform in all series of the experiment. Comparable data were also obtained in a replicate experiment despite the differences in season at which performed. Furthermore, in a previous study with buckwheat (**17**) employing widely varying root and shoot temperatures and a nutrient solution of different composition, similar ontogenetic fluctuations were noted. BAKHUYZEN (**3**), DENNISON (**13**) and SMIRNOV (**35**) have observed similar trends in the rates of water intake during the life cycle of wheat and tobacco. The similarity of data for the gherkin to those of other plants seems to justify the conclusion that sys-

temic changes in absorption of inorganic nutrients and the altered internal distribution of these and organic materials are definitely correlated with if not actually determined by the onset of the reproductive processes (**3, 5, 21, 22, 26, 27, 41**). Thus, for the gherkin as well as for many other plants, specific changes in the rates of water and nutrient absorption, alterations of the substrate as well as certain morphological responses seem to be characteristic of specific stages of ontogeny, even under widely varying environmental conditions. The fact that plants in the present experiment were grown at uniform levels of nutrient supply throughout the life cycle lends support to the conclusion that certain of the observed responses are closely correlated if not conditioned by internal regulatory factors of the plants themselves.

Although the term photoperiodism is generally associated with the flowering response, GARNER and ALLARD (**15**) have indicated that there are also important vegetative responses due to the photoperiod. Recognition of some of these vegetative responses is greatly facilitated by the use of a photoperiodically indeterminate species whose flowering is relatively independent of the day length. In the present instance, gherkin plants receiving 16 hours of illumination daily excelled in most growth responses, judged on the basis of yield. Reduction in total weight under short-day illumination coincides with the observations of several investigators (**11, 13, 39**) working with a variety of photoperiodic and indeterminate plants. A larger proportion of the total carbohydrate content of the short-day plants was in the insoluble form. Comparable responses for tobacco and chrysanthemum were observed by DENNISON (**13**) and GRAINGER (**16**) respectively under short-day conditions. SCULLY *et al.* (**34**) have shown that photoperiodic control of bulb development which is a vegetative response in Allium is not necessarily associated with floral initiation. The differences in growth under contrasted day length indicates that the photoperiodic classification of EGUCHI (**14**) has considerable merit at least for the so-called "day neutral" type of plants. Finally it may be noted that, though flower primordia were initiated simultaneously in the gherkin under long- and short-day conditions, many more flowers were formed on short-day plants.

Summary

Certain features of the ontogenetic cycle of *Cucumis anguria* grown under greenhouse conditions in gravel cultures maintained at uniform levels of water and nutrient supply were traced from germination to maturity. Growth and development were compared in high- and low-nitrogen plants in both long and short day (photoperiods of eight and 16 hours). The following responses were observed:

1. Stems of high-nitrogen, long-day plants attained a greater length than stems of high-nitrogen, short-day plants, although rapid extension of the main axis was delayed until late in anthesis of plants under long-day

conditions. On the other hand, at low-nitrogen levels, stem length of short-day plants exceeded that of the long-day plants throughout.

2. Plants in the eight-hour photoperiod produced more nodes and leaves but a smaller leaf area and smaller root systems than plants in the 16-hour day length.

3. The period between the origin of flower primordia and syngamy was characterized in all series by diminished differentiation of new vegetative organs. During this period, however, there occurred the maximum gains in leaf area and internode elongation. On the whole, it was also a period of low water and salt intake and of fairly stable but low pH of the nutrient substrates.

4. Resurgence of vegetative differentiation accompanied by increased water and nutrient absorption was quite pronounced in the early stages of fruit formation.

5. Flower bud inception occurred simultaneously in all experimental groups irrespective of the variables employed. The peak of flower production occurred 15 days earlier in plants exposed to eight-hour day length and about 50 per cent. more flowers were formed in short- than in the long-day plants. More pistillate flowers were produced by the high-nitrogen plants in both photoperiods.

6. Long-day plants consistently elaborated greater fresh and dry weights than short-day plants. Long day favored root growth. Low nitrogen depressed growth of shoots more than roots of the gherkin.

7. Most of the ash was found in the shoots of plants in both photoperiods but low nitrogen supply was correlated with marked accumulation of ash in the roots. After full bloom, short-day plants showed a net decrease in ash content in contrast to a net gain of plants in long day.

8. Long-day, high-nitrogen plants had elaborated greater actual amounts of carbohydrates than short-day high-nitrogen plants at both sampling dates. When subjected to low-nitrogen nutrition, the long-day plants also contained more carbohydrates than short-day plants at anthesis, but the converse relationship existed at maturity.

9. In general, short-day plants at both levels of nitrogen supply displayed a higher percentage of total nitrogen throughout ontogeny. Total nitrogen increased progressively with age in all groups except in low-nitrogen, long-day plants. Most of the total nitrogen was in the soluble form in all groups from anthesis to maturity in all variables except in mature long-day high-nitrogen plants.

10. The data suggest that in plants grown at uniform levels of nutrient supply, certain aspects of ontogeny are closely correlated with specific stages of development and perhaps conditioned by them as inherent regulatory factors.

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BRIEF PAPERS

EFFECT OF 2,4-D ON YIELDS OF MAIZE IN THE SUCCEEDING GENERATION AFTER TREATMENT[1]

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Received April 6, 1949

The use of 2,4-dichlorophenoxyacetic acid for weed control in hybrid seed corn crossing fields has not been recommended generally. The reluctance to use the herbicide in seed fields has been prompted by fear of damage to the valuable seed corn crop in the year in which the treatment is made, and the possibility of deleterious effects in seed produced on treated parental plants and in plants of the succeeding generation.

A study was inaugurated at Ames, Iowa in 1947 to answer some of the questions regarding the effect of 2,4-D on seed corn production. Inbred lines of corn were used to obtain the desired genetic uniformity. The effects of an aqueous spray of the sodium salt of 2,4-D on the parent plants, seed yields, seed germination, and seedling vigor have been reported previously (**1**). The present report gives the results for the 1948 yield trial grown from single-crossed seed produced on inbred plants sprayed with 2,4-D in 1947.

Materials and methods

In 1947 four inbred lines of corn, M14, WF9, Os420, and, W22 were sprayed with an aqueous solution of 400 p.p.m. or 1600 p.p.m. of the sodium salt of 2,4-D at three stages of plant development: (1) young plants with six to eight leaves, (2) at the time of tassel and ear shoot emergence, and (3) ten days after pollination. A separate group of plants was used for each of the six treatments and a seventh group was left for an unsprayed check. Hand pollinations were made to produce single-crossed seed of all possible combinations among the parent lines, including reciprocals. Data were obtained on the visual effects of 2,4-D on the parent plants and on the seed yields, and the single-crossed seed was tested for germination and seedling vigor (**1**).

Single-crossed seed representing 49 of the 84 possible variety-treatment combinations was planted May 10, 1948 in a 7×7 lattice square design with four replications at Ames, Iowa. Plots were 2×10 hills in size. Five kernels were planted per hill and later thinned to three plants per hill. The plots were harvested November 2, 1948.

[1] Journal paper No. J-1194 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project No. 163, in cooperation with the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture.

Experimental results

A favorable corn growing season resulted in good yields of corn with low moisture contents. The mean moisture content for the experiment

TABLE I

MEAN YIELDS IN BUSHELS PER ACRE OF CORN AND STAND PERCENTAGES FOR 12 SINGLE CROSSES GROWN IN 1948 AT AMES, IOWA. THE SEED FOR THE YIELD WAS PRODUCED ON INBRED PLANTS WHICH WERE EITHER UNSPRAYED FOR CONTROLS OR SPRAYED WITH 2,4-D IN 1947

SINGLE CROSS	CHECK NOT SPRAYED	TIME OF TREATMENT—1947					
		6-8 LEAVES		TASSEL EMERGENCE		10 DAYS AFTER POLLINATION	
		RATE P.P.M.		RATE P.P.M.		RATE P.P.M.	
		400	1600	400	1600	400	1600
W22 × M14	95.1	93.1	94.0	95.2	.....	97.7	106.9
	(92.1)*	(92.1)	(83.8)	(88.8)	.....	(90.0)	(93.8)
M14 × W22	98.4	93.9	.....	100.3	.....	94.5	86.6
	(91.3)	(89.2)	.....	(92.5)	.....	(85.4)	(79.2)
Os420 × WF9	120.3	.....	.....	112.8	.....	.....	.....
	(87.9)	.....	.....	(77.9)	.....	.....	.....
WF9 × Os420	127.7	.....	.....	133.0	.....	.....	.....
	(80.8)	.....	.....	(91.3)	.....	.....	.....
WF9 × M14	106.9	93.7	87.6	109.9	.....	111.8	105.6
	(82.5)	(39.6)	(63.8)	(88.8)	.....	(88.3)	(80.0)
M14 × WF9	116.1	117.5	112.8	111.3	.....	114.6	116.3
	(92.1)	(86.7)	(86.3)	(89.2)	.....	(88.8)	(89.2)
W22 × Os420	110.5	114.9	.....	106.0	.....	117.2	122.0
	(88.3)	(89.2)	.....	(75.4)	.....	(87.1)	(91.3)
Os420 × W22	114.9	92.4	.....	104.4	104.0	101.0	115.7
	(92.5)	(80.4)	.....	(82.1)	(85.0)	(84.2)	(91.7)
M14 × Os420	119.0	128.7	.....	110.6	.....	.....	.....
	(92.9)	(92.1)	.....	(85.4)	.....	.....	.....
Os420 × M14	118.1	93.7	.....	101.6	.....	.....	.....
	(87.5)	(71.7)	.....	(78.8)	.....	.....	.....
W22 × WF9	102.2	.....	.....	111.9	.....	.....	.....
	(79.2)	.....	.....	(82.1)	.....	.....	.....
WF9 × W22	118.7	119.3	.....	119.7	.....	.....	.....
	(86.3)	(92.1)	.....	(87.1)	.....	.....	.....
Mean (6)**	107.0	100.9	.....	104.5	.....	106.1	108.9
	(89.8)	(79.5)	.....	(86.1)	.....	(87.3)	(87.5)
Mean (12)**	112.3	.....	.....	109.7	.....	.....	.....
	(87.8)	.....	.....	(85.0)	.....	.....	.....
Mean (9)**	110.9	105.2	.....	106.6	.....	.....	.....
	(89.5)	(81.5)	.....	(85.3)	.....	.....	.....
Mean (3)**	106.0	101.4	98.1	105.5	.....	108.0	109.6
	(88.9)	(72.8)	(78.0)	(88.9)	.....	(89.0)	(87.7)

Least significant difference between any two variety-treatment combinations in body of table = 13.7 bushels.

* Numbers in parentheses are the stand percentages.

** Means of all single crosses receiving the same treatments. The number of single crosses in each mean is given in parentheses.

was 16.0 per cent with a range of 19.2 to 14.0 per cent. Differences in

moisture content were not associated with treatment. Lodging, ear height, and percentage of dropped ears were not affected by the treatments of the previous year.

Mean yields and stand percentages for the 49 variety-treatment combinations are presented in table I and the analysis of variance for the field weights in table II. As a result of limited seed production on the more

TABLE II

ANALYSIS OF VARIATIONS OF FIELD WEIGHTS

SOURCE OF VARIATION	DEGREES OF FREEDOM	MEAN SQUARE
Replications	3	3.8
Rows	24	93.4**
Columns	24	7.8
Entries (adjusted)	48	67.1
Single crosses	11	216.8
Treatments	6	54.3
Single crosses × treatments	31	16.4**
Error	91	7.7

** Significant at the 1 per cent. level of probability.

severely injured plots in 1947, it was not posible to yield test all of the 84 possible treatment combinations. The yield results presented here are from the less damaged plots as measured by seed production in 1947. The number of direct comparisons are somewhat limited by the disproportionate sub-class frequencies.

A series of means are presented at the bottom of table I. The values listed for mean (**6**) represent the mean for the six single crosses sprayed with 2,4-D 10 days after pollination and the means for the same six crosses receiving other spray treatments. The remaining means listed were obtained in a similar manner.

The interaction of single crosses × treatments was highly significant (table II) indicating that all single crosses did not respond similarly to the 2,4-D treatments. The larger yield reductions occurred as a result of 2,4-D sprays applied in the six-eight leaf stage the previous year. Sprays applied 10 days after pollination produced only slight or no reductions in yield. When the interaction single crosses × treatments is used as the error term the differences in yield among the spray treatments were significant at the 5 per cent. level.

Stands ranged from 93.8 per cent. to 39.6 per cent. with a mean of 85.2 per cent. for the experiment. Adjustments in yield were made for missing hills, but no adjustments were made for hills having one or two plants per hill. The low stand percentages were, in general, associated with 2,4-D treatments and lower yields of corn. It is highly probable that greater yield reductions would have occurred from 2,4-D treatments if excessive

planting and thinning had not been practiced in an attempt to insure equal stands of corn. The previous report (1) showed a higher percentage of weak seedlings in the seed produced on the sprayed inbred plants.

Summary

Single-crossed seed produced on unsprayed inbred plants and on plants sprayed with 2,4-D at three stages of growth in 1947 was tested for yielding ability in 1948.

On the basis of one year's data, the results indicate that in some cases there was a carry-over effect on the succeeding crop after spraying corn plants with 2,4-D. Significant reductions in yield of grain occurred in some of the single crosses, while others were apparently not affected. Yield reductions were more serious as a result of 2,4-D sprays applied at the six to eight leaf stage the previous year than were those applied at the later stages of plant development. Losses in yield were associated with reductions in stand.

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BUREAU OF PLANT INDUSTRY, SOILS, AND AGRICULTURAL ENGINEERING
AGRICULTURAL RESEARCH ADMINISTRATION
UNITED STATES DEPARTMENT OF AGRICULTURE

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1. ROSSMAN, ELMER C., and STANIFORTH, DAVID W. Effects of 2,4-D on inbred lines and a single cross of maize. Plant Physiol. **24**: 60–74. 1949.

NOTES

Annual Election. The twenty-sixth annual election of the American Society of Plant Physiologists has resulted in the election of the following officers for 1949–50:

President, Dr. James Bonner, California Institute of Technology, Pasadena, California.

Vice-President, Dr. C. H. Wadleigh, U. S. Regional Salinity Laboratory, Riverside, California.

Secretary, Dr. T. C. Broyer, University of California, Berkeley, California.

Member of the Executive Committee, Dr. H. A. Spoehr, The Carnegie Institution of Washington, Stanford University, California.

Member of the Editorial Board, Dr. E. J. Kraus, Oregon State College, Corvallis, Oregon.

The constitutional amendment providing that stipulations as to the amount of dues paid by a given member should be stricken from the Constitution and transferred to the by-laws was carried by a 10 to 1 margin.

The Executive Committee has voted approval of holding the 1950 annual meeting in affiliation with the A.I.B.S. at Columbus, Ohio, on September 11, 12, and 13, 1950.

American Society of Plant Physiologists Annual Meeting, 1949. New York City. The American Society of Plant Physiologists is meeting this year with the American Association for the Advancement of Science and affiliated societies at New York City, December 26 to 31, inclusive. The A.S.P.P. sessions will be held December 27 to 29, inclusive, in the Hotel McAlpin. Reservations for lodging should be made with the New York Convention Bureau, New York City. All A.S.P.P. members are asked to register formally with the A.A.A.S. in order that the Society will be assisted in offsetting meeting expenses.

Earl Long Overholser. Members of the American Society of Plant Physiologists will be grieved to learn of the sudden death of Dr. Earl. L. Overholser from a heart attack at Blacksburg, Virginia, April 18, 1949. Dr. Overholser was born in Harrisonville, Missouri in 1888. He received a B.S. degree in horticulture at the University of Missouri in 1913 and the M.S. in 1914. He received his Ph.D. degree in horticulture from Cornell University in 1926. Dr. Overholser held faculty positions at the University of Missouri, Cornell University, University of California and State College of Washington before going to Virginia Polytechnic Institute where he was head of the Department of Horticulture at the time of his death.

Edwin C. Miller. The American Society of Plant Physiologists records with sorrow the death, at the age of 70 years, of Dr. E. C. Miller, professor emeritus of plant physiology at Kansas State College and formerly plant physiologist of the Kansas Agricultural Experiment Station, Manhattan, Kansas. Dr. Miller died August 2 in Superior, Nebraska of cerebral thrombosis. Dr. Miller was born near Baltimore, Ohio, in 1878. He received the B.S. degree in 1904 and the A.B. degree in 1906 from the old National Normal University at Lebanon, Ohio. Then followed an A.B. and the Ph.D. from Yale University in 1907 and 1910, respectively. He served as president of the American Society of Plant Physiologists and subsequently as a member of the Editorial Board of Plant Physiology. Dr. Miller is survived by the widow, Zina Della Miller, a son, Jordan Miller of Lowell, Massachusetts, and a daughter, Mrs. Philip R. Henderson of Superior, Nebraska.

Handbook of Biological Data. The American Institute of Biological Sciences, a component of the Division of Biology and Agriculture of the National Research Council, has set up a committee to prepare a "Handbook of Biological Data." Collection of the material is underway. The book will present the basic quantitative data in the biological sciences, with their applications in medicine, agriculture and industry. It will be written for the student as well as for the teacher, the investigator and the practitioner. Above all, it is intended that the book shall be clear in presentation and easy to consult. Cooperation in bringing the plan to successful fruition is solicited by the committee. Authoritative quantitative data are needed—as well as qualitative data that are susceptible of tabular or other concise presentation. The Committee is interested in including material from all branches of biology: tabular material compiled in each field issued to students and tables in texts or in reprints that are used for occasional reference in research or practice.

Those willing to join the list of contributors to the Handbook should mail the Committee tables to be included, sections of tables, or even single well established values that seem suitable, marked reprints, or references to the publications. If a table is only an idea at present, but one that should be given substance and included in the book, the Committee should be mailed a plan of the table with a suggestion of someone competent to compile it. Address the Executive Secretary, Committee on the Handbook of Biological Data, 2101 Constitution Avenue, Washington 25, D. C. The Committee requests that suggestions be sent to our representative, Dr. David R. Goddard, Professor of Botany, University of Pennsylvania, Philadelphia, Pennsylvania.

Advances in Enzymology and Related Subjects of Biochemistry, Volume IX. Edited by F. F. Nord. Interscience Publishers, Inc., 215 Fourth Avenue, New York 3, New York. 760 pages, 1949. $9.00.

This volume, the latest addition to the well known series, includes the following: Some Aspects of Reversible Step Reactions by L. Michaelis; Kinetics of Biological Reactions with Special Reference to Enzymic Processes by A. E. Stearn; Photochemistry of Enzymes, Proteins and Viruses by A. Douglas McLaren; The Nature of Viruses by M. A. Lauffer, W. C. Price and A. W. Petre; Nitrogen Metabolism of Higher Plants by H. E. Street; Chemistry and Enzymology of Nucleic Acids by F. Schlenk; Pathways of Acid Formation in *Aspergillus niger* and in Related Molds by T. K. Walker; Principles of Enzymic Histo- and Cyto-chemistry by David Glick; Enzyme Activity in Frozen Vegetable Tissue by M. A. Joslyn; and Industrial Biosynthesis. Part I. Fats, by Albert Hesse. The current volume contains both author and subject indices and also a cumulative index for volumes one to nine inclusive.

Annual Review of Biochemistry, Volume XVIII. Edited by J. MURRAY LUCK. Annual Reviews, Inc., Stanford, California. 739 pages, 1949. $6.00.

This volume contains twenty-three sections of which the following will be of special interest to plant scientists: Biological Oxidations by P. W. Preisler and F. E. Hunter, Jr.; Proteolytic Enzymes by E. L. Smith; Nonoxidative, nonproteolytic Enzymes by K. Myrback; Carbohydrate Chemistry by D. J. Bell; Chemistry of the Lipids by J. A. Lovern; Chemistry of Amino Acids and Proteins by H. P. Lundgren and W. H. Ward; Nucleoproteins, Nucleic Acids and Derived Substances by J. N. Davidson; Metabolism of the Lipids by A. L. Lehninger; Metabolism of Proteins and Amino Acids by A. Neuberger; Intermediary Metabolism of Phosphorus Compounds by F. Lipmann and N. O. Kaplan; Carbohydrate Metabolism by H. G. Wood and V. Lorber; Mineral Metabolism by F. J. McClure; Chemistry of the Hormones by A. Wettstein and F. Benz; Fat Soluble Vitamins by P. L. Harris; Water Soluble Vitamins by E. L. R. Stokstad and T. H. Jukes; Metabolic Inhibitors by R. J. Winzler; Chemistry of Antibiotics by O. Wintersteiner and J. D. Dutcher; Nitrogenous Constituents of Plants by A. G. McCalla; Organic Acids of Plants by T. A. Bennet-Clark; Mineral Nutrition of Plants, by C. H. Wadleigh. The volume contains comprehensive subject and author indices.

Physics and Chemistry of Cellulose Fibres. P. H. HERMANS. Elsevier Publishing Company, Inc., 215 Fourth Avenue, New York 3, New York. 534 pages, 1949. $9.50.

This book consists of three parts. Part I deals with the molecular and crystalline structure of cellulose and with the behavior of chain molecules in solution and in the gel state. Part II deals with the morphology and the physical and chemical properties of natural and artificial cellulose fibres. A very extensive and fundamental discussion of the swelling and deformation processes in regenerated cellulose model filaments is given in

Part III. Here the author emphasizes the analogies and differences existing between the swollen cellulose gel and other rubber-like materials. The many references to modern literature on this subject and the extensive subject index constitute a most valuable feature of the book.

Pre-Medical Physical Chemistry. F. A. MATSEN, JACK MYERS, and NORMAN HACKERMAN. The MacMillan Company, 60 Fifth Avenue, New York, New York. 335 pages, 1949. $4.75.

In this text the authors have set forth the principles of physical chemistry of interest to physiology students. The development of topics has been designed to introduce students to the principles and to encourage recognition of those principles operating in biological phenomena. With this in mind, biological applications have frequently been cited, and the space allotted to topics is determined on a basis of biological importance. Additional references are listed to guide more extensive reading on any topic.

Among the topics included are: atomic structure and isotopes, molecular structure and intermolecular forces, properties of matter in the different states, elementary kinetics and equilibria, oxidation-reduction, principles concerned with pH, surface phenomena, colloidal properties and biological energy exchange.

Practical Plant Anatomy. ADRIANCE S. FOSTER. Second Edition. D. Van Nostrand Company, Inc., 250 Fourth Avenue, New York, New York. 228 pages, 1949. $3.00.

The purpose of this volume is to provide for the student a means of joining the study of laboratory material with modern theory and interpretation. Extended treatment is given to meristems, sclerenchyma, the structure and ontogeny of the primary vascular tissues and the development and anatomy of the stem, leaf and root. The volume is largely confined to the study of the "vegetative body" of the seed plants, as in the first edition. A new exercise dealing with the structure and ontogeny of laticiferous tubes has however been added as well as new material on the topic of floral histogenesis.

Les Champignons Parasites des Plantes Cultivées. G. VIENNOT-BOURGIN. Masson et. Cie., 120, Boulevard Saint-Germain, Paris—6e. 2 Volumes, 1851 pages, 1949. 6000 francs.

This work constitutes the most comprehensive summary of the fungi causing plant disease which has appeared since the publication of Sorauer's "Handbuch." Some idea of its scope may be gathered from the 175 page bibliography in which over 6000 references are cited. The arrangement is strictly taxonomic and the classification adopted is conservative. Anyone

familiar with the treatment of the fungi in the first edition of Engler and Prantl will have no difficulty in comprehending the sequence followed. Emphasis throughout is on the morphology of the fungi and the treatment of the crops attacked by them. The physiological aspects discussed are chiefly those concerned with the effect of temperature and moisture on sporulation and infection; other topics are introduced in connection with particular fungi and references given to sources of further information. The 720 illustrations are well selected and helpful although the soft paper used has affected the clearness of some of the half-tones. A single index combines references to fungi and hosts under Latin names and to diseases under French and sometimes German and English names. The volumes will undoubtedly prove extremely useful in any laboratory where plant disease fungi are studied.

Précis de Microscopie, Collection des Précis Médicaux. M. LANGERON. Seventh edition. Masson Edit., Paris. 1430 pages, 1949. 2600 francs.

The seventh edition of Langeron's well known handbook of great interest to plant physiologists, deals with histochemistry, microchemistry and chromatic analysis of tissues. The author indicates the applicability and limitations of these procedures, pointing out that chromatic analysis aims toward the detection of certain compounds through specific staining while histochemistry aims at locating reactive chemical groups or structures at their natural site in the tissues and that microchemistry achieves the identification of chemical constituents, but at the price of disruption of the histological and cytological structures. Critical evaluation is given of techniques for determination of intracellular pH, rH, for the study of enzymes in the tissues (notably peroxidases, phenolases, hydrolases, phosphatases, lipases . . .), for the localization and identification of cations and anions, of proteides (notably polypeptides involving — SH groups) of nucleo-proteins and nucleotides.

Part III discusses special problems of plant histology and cytology. The general topics are discussed in the light of recent publications up to and including 1948 with emphasis on their relation to physico-chemical problems of fundamental interest in plant physiology.

Dynamics of Vegetation. Selections from the writings of Frederic E. Clements. Compiled and edited by B. W. ALLRED and EDITH S. CLEMENTS. The H. W. Wilson Company, 950 University Avenue, New York 52, New York. 296 pages, 1949. $3.75.

This handsomely illustrated book emphasizes the dynamic side of plant ecology. The editors have selected papers from Dr. Clements' writings which are of a practical nature. The subject matter is tied up with human affairs in agriculture, forestry, range management, and conservation. This book fulfills the need for collected readings on this subject.

Field Manual of Plant Ecology. FRANK C. GATES. McGraw-Hill Book Company, Inc., 330 West 42nd Street, New York, New York. 137 pages, 1949. $3.00.

This manual, based upon thirty-two years of teaching experience, clearly describes procedures and feasible materials for field work in ecology. Suggestions to both teacher and student have been included. The author's thorough appreciation of the problems of such instruction is evidenced by suggestions regarding clothing, useful personal equipment, and for minimizing the discomforts caused by poison ivy and insect pests.

French Bibliographical Digest, Number 2. The Cultural Division of the French Embassy, 934 Fifth Avenue, New York 21, New York. 85 pages, 1949.

This volume contains a bibliography of the most significant books on animal and plant biology, zoology and botany published in France between 1940 and 1948. The issue complements volume No. 1 published in March 1949 which presented a bibliography of works concerning general biology, microbiology, biochemistry, and experimental biology. The reviews appearing herein have been taken from the following publications: The Quarterly Review of Biology, Revue Scientifique, Bulletin de l'Institut Pasteur, Bulletin Critique du Livre Francais.

The Periodic Partial Failures of American Cottons; Their Causes and Remedies. R. H. DASTUR. Second Edition. Indian Central Cotton Committee, Bombay, India. 172 pages, 1949. Rs 6.

This volume contains the main findings of an investigation on the partial failures of the Punjab-American cottons in the Punjab and Sind. The investigations deal with two important physiological diseases, bad opening of bolls or *tirak* and the red leaf disease, which were prevalent in these two most important American cotton growing tracts in India. The book represents the results of years of patient scientific work honestly and competently carried out.

Fifty Years of Plant Physiology. TH. WEEVERS. Scheltema et. Holkema's Boekhandel En Uitgevers Maatschappij N. V., Rokin 74–75, Amsterdam. 308 pages, 1949.

This work contains a review of the development of plant physiology from 1895 to 1945. Chapter headings include: Introduction, comprising an outline of the book and a short summary of plant physiology to 1895: Respiration; Imbibition and Diffusion; Movement of Water; Mineral Nutrition; Translocation; Metabolism of Heterotrophic Plants; Growth, Phototropism, and Geotropism; Significance of Internal and External Factors in Growth and Differentiation; Tropisms and Nasties; Locomotor Movements. The book also includes an author index and bibliography and subject index.

Experiments in Soil Bacteriology. O. N. ALLEN. Burgess Publishing Company, 426 South Sixth Street, Minneapolis 15, Minnesota. 126 pages, 1949. $2.50.

This manual is designed for a one semester course in soil bacteriology for students on the senior and graduate level whose major interests are in agricultural bacteriology, soils, chemistry and agronomy. The manual consists of 40 exercises and each experimental section is prefaced by a list of selected comprehensive references.

Dennis R. Hoagland. Dr. D. R. Hoagland, 65, professor and chairman of the Division of Plant Nutrition, College of Agriculture, University of California, died September 5 after a long illness.

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